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Research

Recent Research on EMF and Health Risk, Seventeenth report from SSM's Scientific Council on Electromagnetic Fields, 2022

2024:05

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SSM perspektiv

Bakgrund

Strålsäkerhetsmyndighetens (SSM) Vetenskapliga råd för elektromagnetiska fält övervakar aktuell forskning om potentiella hälsorisker i relation till exponering för elektromagnetiska fält och ger myndigheten råd om bedömning av möjliga hälsorisker. Rådet ger vägledning när myndigheten måste ge ett yttrande om politiska frågor där vetenskaplig utvärdering är nödvändig. Rådet är skyldigt att årligen lämna in en skriftlig rapport om den aktuella forskningen och kunskapsläget.

Detta är en konsensusrapport. Det innebär att alla medlemmar i det Vetenskapliga rådet håller med om den fullständiga rapporten. Detta ökar styrkan i de givna slutsatserna.

Rapportens främsta mål är att täcka föregående års forskning inom området elektromagnetiska fält (EMF) och hälsa, men också att placera detta i sammanhanget av nuvarande kunskap. Rapporten ger myndigheten en översikt och utgör en viktig grund för riskbedömning.

Resultat

Denna rapport granskar studier om elektromagnetiska fält (EMF) och hälsorisker, publicerade från januari 2021 och fram till och med december 2021. Rapporten är den sjuttonde i en serie årliga vetenskapliga översikter som successivt diskuterar och bedömer relevanta nya studier och placerar dessa i sammanhanget av tillgänglig information. Rapporten täcker olika områden av EMF (statiska, lågfrekventa, intermediära och radiofrekventa fält) samt olika typer av studier såsom biologiska, mänskliga och epidemiologiska studier. Resultatet kommer att vara en gradvis utveckling av en hälsoriskbedömning av exponering för EMF.

Inga nya etablerade orsakssamband mellan exponering för EMF och hälsorisk har identifierats.

De studier som presenteras i denna rapport löser inte om det konsekvent observerade sambandet mellan exponering för ELF magnetfält (ELF-MF) och barncancer inom epidemiologi är kausalt eller inte.

Ny forskning om hjärntumörer och mobiltelefonanvändning är i linje med tidigare forskning som mestadels antyder en frånvaro av risk. Sköldkörteln är potentiellt mycket exponerad under mobiltelefonsamtal, men det har hittills utförts lite forskning om sköldkörtelcancer.

När det gäller studier på djur är det svårt att dra generella slutsatser annat än att under vissa omständigheter observeras vissa effekter av RF-EMF-exponering på försöksdjur. Observationerna av ökad oxidativ stress som rapporterats i tidigare SSM-rapporter fortsätter att påträffas, vissa till och med under nuvarande referensnivåer. Oxidativ stress är en naturlig biologisk process som ibland kan vara involverad i patogenes, men under vilka omständigheter oxidativ stress på grund av svag bestrålning av radiofrekventa fält kan påverka människors hälsa återstår att undersöka.

Det är värt att notera att nya studier har visat att uppfattningströsklarna är lägre under hybrida exponeringsförhållanden än under endast DC- eller AC-fältexponering.

Trots den ökande användningen av applikationer inom det intermediära frekvensområdet (IF) av det elektromagnetiska spektret (300 Hz-10 MHz) är den vetenskapliga utvärderingen av potentiella hälsorisker inom det intervallet knapphändig. De få studier som identifierats av rådet på detta område har dock inte indikerat några hälsorisker under nuvarande referensnivåer.

Den årliga rapporten inkluderar också ett avsnitt där studier som saknar tillfredsställande kvalitet har listats. I år, liksom förra året, har många studier uteslutits på grund av dålig kvalitet (se bilaga). Ur ett vetenskapligt perspektiv är studier av dålig kvalitet irrelevanta. De är också ett slöseri med pengar, mänskliga resurser och i många fall försöksdjur.

Slutsatser

Resultaten av forskningsöversikten ger ingen anledning att ändra några referensnivåer eller rekommendationer på området. Emellertid visar observationer av biologiska effekter på djur på grund av svag exponering för radiovågor tydligt vikten av att bibehålla försiktighetsprincipen enligt den svenska miljöbalken.

SSM:s rekommendation om handsfree för mobiltelefonsamtal kvarstår även om trenderna för gliomincidenser inte ger stöd för en ökande risk orsakad av mobiltelefonradiovågs exponering. Dock motiverar observerade biologiska effekter och osäkerheter angående möjliga långsiktiga effekter försiktighet.

Inga nya fynd som tydligt förändrar misstanken om ett orsakssamband mellan svaga lågfrekventa magnetfält och barncancer har framkommit i rapporten. De svenska myndigheternas rekommendation att generellt begränsa exponeringen för lågfrekventa magnetfält på grund av den observerade ökningen av barncancer nära kraftledningar förblir oförändrad.

Projektinformation

Kontaktperson SSM: Karl Herlin
Referens: SSM2024-2038 / 4530071

SSM perspective

Background

The Swedish Radiation Safety Authority's (SSM) Scientific Council on Electromagnetic Fields monitors current research on potential health risks in relation to exposure to electromagnetic fields and provides the authority with advice on assessing possible health risks. The Council gives guidance when the authority must give an opinion on policy matters when scientific testing is necessary. The council is required to submit a written report each year on the current research and knowledge situation.

This is a consensus report. This means that all members of the Scientific Council agree with the complete report. This increases the strength of the given conclusions.

The report has the primary objective of covering the previous year's research in the area of electromagnetic fields (EMF) and health but also to place this in the context of present knowledge. The report gives the authority an overview and provides an important basis for risk assessment.

Results

This report reviews studies on electromagnetic fields (EMF) and health risks, published from January 2021 up to and including December 2021. The report is the seventeenth in a series of annual scientific reviews which consecutively discusses and assesses relevant new studies and put these in the context of available information. The report covers different areas of EMF (static, low frequency, intermediate and radio frequency fields) and different types of studies such as biological, human and epidemiological studies. The result will be a gradually developing health risk assessment of exposure to EMF.

No new established causal relationships between EMF exposure and health risk have been identified.

The studies presented in this report do not resolve whether the consistently observed association between ELF magnetic field (ELF-MF) exposure and childhood leukaemia in epidemiology is causal or not.

New research on brain tumours and mobile phone use is in line with previous research suggesting mostly an absence of risk. The thyroid gland is potentially highly exposed during mobile phone calls but little research on thyroid cancer has been conducted so far.

Concerning studies on animals, it is difficult to draw general conclusions other than that under certain circumstances some effects from RF-EMF exposure are observed in experimental animals. The observations of increased oxidative stress reported in previous SSM reports continue to be found, some even below current reference levels. Oxidative stress is a natural biological process that can sometimes be involved in pathogenesis, but under what circumstances oxidative stress due to weak radio wave exposure may affect human health remains to be investigated.

It is notable that new studies revealed that perception thresholds are lower in hybrid exposure conditions than in DC or AC field exposure alone.

Despite the increasing use of applications in the intermediate frequency (IF) range of the electromagnetic spectrum (300 Hz-10 MHz), scientific evaluation of potential health risks in that range is scarce. However, the few studies identified by the council in this area have not indicated any health effects below current reference levels.

The annual report also includes a section where studies that lack satisfactory quality have been listed. This year, as well as last year, many studies have been excluded due to poor quality (see appendix). From a scientific perspective, studies of poor quality are irrelevant. They are also a waste of money, human resources and, in many cases, experimental animals.

Conclusions

The results of the research review give no reason to change any reference levels or recommendations in the field. However, the observations of biological effects in animals due to weak radio wave exposure clearly show the importance of maintaining the Swedish Environmental Code precautionary thinking.

SSM´s hands-free recommendation for mobile phone calls remains even though trends of glioma incidences do not provide support for an increasing risk caused by mobile phone radio wave exposure. However, observed biological effects and uncertainties regarding possible long term effects justify caution.

No new findings that clearly change the suspicion of a causal link between weak low-frequency magnetic fields and childhood leukaemia have emerged in the report. The Swedish authorities' recommendation to generally limit exposure to low frequency magnetic fields due to the observed increased incidence of childhood leukaemia close to power lines remains unchanged.

Project information

Contact person SSM: Karl Herlin
Reference: SSM2024-2038 / 4530071

Recent Research on EMF and Health Risk

Seventeenth report from SSM's Scientific Council on Electromagnetic Fields, 2022

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Preface

The Swedish Radiation Safety Authority´s scientific Council for electromagnetic fields (EMF) and health was established in 2002. The Council´s main task is to follow and evaluate the scientific development and to give advice to the authority. In a series of annual reviews, the Council consecutively discusses and assesses relevant new data and put these in the context of available information. The result will be a gradually developing health risk assessment of exposure to EMF. The Council presented its first report in 2003. A brief overview of whether or how the evidence for health effects has changed over the first decade of reports was included in the eleventh report. The present report is number seventeen in the series and covers studies published from January 2021 up to and including December 2021.

The composition of the Council that prepared this report has been:

Clemens Dasenbrock, Dr. med. vet., professor, toxicology/experimental oncology, Uetze, Germany

Aslak Harbo Poulsen, PhD, epidemiology, Danish Cancer Society, Copenhagen, Denmark

Karl Herlin, MSc, Physics, Sweden (scientific secretary)

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Declarations of conflicts of interest are available at the Swedish Radiation Safety Authority.

Stockholm/ Utrecht in January 2023

Anke Huss

Chair

Executive summary

Static fields

Epidemiological studies

Last years' summary on static field (or MRI) exposure and health effects is essentially unchanged: Research on occupational MRI exposure or other strong static field exposures is scarce and underlying mechanisms for occasionally observed associations are unclear. Two new studies published in 2021 do not challenge that observation. Given that exposure to static fields from MRI is relatively high, and the number of occupationally exposed persons is increasing, more systematic and comprehensive research on this topic is warranted.

Human studies

Detection thresholds for direct current (DC), alternating current (AC), and hybrid electric fields have been systematically investigated in a carefully designed large scale study, which was preceded by a pilot study to identify relevant environmental and experimental factors. The studies revealed that perception thresholds are lower in hybrid exposure conditions than in DC or AC exposure alone. This research adds valuable information for a discussion of the acceptability of sensory perception of EF and the so far not existing reference levels and recommendations for hybrid exposure, which is important for political decision-making processes around the construction of high voltage DC power and hybrid power lines.

Animal studies

Strong magnetic fields in the range of 16.4 T affected motor coordination and balance in rats. Following exposures to 2 -12 T, the mineral element content (Mg, Fe, Zn, Ca, and Cu) in mice' main organs was affected, but no consistent pattern was seen. It is unclear if or how such a finding would also translate into relevance for humans, for example when volunteers are exposed to MRI in research studies.

Static magnetic fields of up to 200 mT were reported to cause some change in glucose metabolism in the brain of rats and could result in anxiety-like behaviours. Addressing potential effects on mice exposed to static electric fields close to ultra-high-voltage direct-current transmission lines (±800 kV), studies did not show long-lasting health effects, especially after exposure stop.

Finally, it was reported that catfish consistently avoid static magnetic fields greater than 20 µT, and after 110 mT exposure in cockroach nymphs, a growth retardation occurred.

As in previous years, all studies reported some but inconsistent effects. This may be due to publication bias. Several research groups use their specific but similar study design over years and report only different endpoints in their successive publications. In addition, most studies are not hypothesis-driven.

In vitro studies

A large number of *in vitro* studies dealing with the effect of static fields have been published in the year 2021 but they were related in most cases to biomedical applications. Only one study was included in the report dealing with the effect of weak static magnetic fields on proliferation and oxidative stress. The results suggest that static fields are able to induce variations in the biological endpoints considered depending on the intensities (between 0.5 and 600 μT).

Extremely low frequency fields

Epidemiological studies

The new studies do not resolve whether the consistently observed association between ELF magnetic field (ELF-MF) exposure and childhood leukaemia in epidemiology is causal or not.

A new study assessing ELF-MF exposure from in-built transformers indicated possible increased risks of melanoma and acute lymphocytic leukaemia in persons who had lived in the higher exposed apartments during childhood. This is an interesting observation that requires follow-up given the small number of exposed cases.

A recent follow-up on an electrical worker cohort on motor neuron disease indicated that possibly more recent exposure to ELF-MF was more relevant than earlier exposures. Electric shocks were not evaluated in this study. The question whether magnetic fields or shocks are underlying the previously observed associations with ALS/ motor neuron disease remains unanswered.

Human studies

Studies published in the current reporting period revealed that low relative humidity (30%) reinforced the perception of AC fields, and that co-exposure of DC and AC electric fields lead to a decrease of the perception threshold compared to either AC of DC alone. Overall, the perception of electric fields was enhanced in the presence of ions. This research adds valuable information for a discussion of the acceptability of sensory perception of EF and the so far not existing reference levels and recommendations for hybrid exposure, which is important for political decision-making processes around the construction of high voltage DC power and hybrid power lines.

One study found that stimulation of the occipital cortex independently from retinal activation can affect phosphene perception, and another study observed some evidence for an activation of the parasympathetic activity by a 15 min 50 Hz exposure.

Animal studies

In contrast to the previous Council reports, only one rodent study was identified. Hyperglycaemia in fed or fasted rats was reported following a single 15 min lasting ELF-MF exposure at 60 Hz and 3.8 mT. Further three studies in honeybees addressing ultra-high voltage (UHV) transmission technology and using ELF-EF (50 Hz, 5 – 34.5 kV/m) confirmed that haemolymph, antioxidant system and behaviour of honeybees are affected when applying 50 Hz EF as low as 5.0 or 11.5 kV/m. In fruit flies exposed to 75 Hz 500 µT ELF-MF, reactive oxygen species were increased, whereas locomotion, eclosion rate and longevity decreased. Following 50 Hz exposures up to 1 T in mosquito larvae severe malformations were detected. Finally, 20 Hz and 50 Hz ELF-MF caused small but significant changes in wingbeat frequency of locusts, and locusts entrained to the exact frequency of the applied ELF-MF.

Overall, various effects were again reported after ELF-MF exposure in different animal models, mostly non-mammalian. Apart from oxidative stress, plausible mechanisms are still unclear.

In vitro studies

In vitro studies published in the year 2021 evaluated the effect of ELF fields on cell proliferation, DNA integrity, ROS production and circadian rhythm. All described effects seem to depend on the basis of the experimental conditions (cell types, exposure duration, frequency, field intensity) and the inconsistent patterns mean that the results are difficult to interpret.

Intermediate fields

Epidemiological studies

A single study on IF-MF exposure and pregnancy outcomes was published in the relevant reporting period; the study does not allow firm conclusions regarding possible health effects from exposure to IF-MF.

Human studies

As for the previous reporting periods, there was no human experimental study in the intermediate frequency range.

Animal studies

In the 20 – 100 kHz range, in total two mouse studies did not result in adverse effects on behaviour and genotoxicity.

In vitro studies

Only one paper has been included in the report, dealing with the exposure of porcine oocytes to evaluate maturation and embryonic development. The authors report that exposure to lower - but not to higher - intensity IF-EMF could interfere with oocyte maturation.

Radiofrequency fields

Epidemiological studies

Several new epidemiological studies did not find an association between mobile phone use and brain tumours. The international MOBI-kids case-control study is by far the largest study on this topic so far. Overall, the study did not find evidence for increased risks. Actually, a trend of decreased risk with increasing mobile phone use was observed. However, in-depth analyses suggest that most likely this observation is biased due to proxy interviews and prodromal symptoms. This illustrates that case-controls studies may have limited potential to detect small risks since they are prone to various types of biases.

A complementary approach is to analyse temporal trends in brain tumour incidence and compare with increases in mobile phone subscriptions. Several such papers have been published on various types of tumours in the head region, which do not indicate an increase in adults paralleled to the uptake of mobile phone use.

A number of systematic reviews postulate an association between mobile phone use and semen quality. However, these reviews were overall based on low quality studies vulnerable to selection bias and often using crude and retrospective exposure assessment methods.

One paper evaluating data from two large prospective cohort studies found little evidence that carrying a mobile phone in a front trouser pocket affected male fertility, although a significant effect in men with a BMI of less than 25 kg/m^2 deserves follow-up investigation. In the same study, carrying a phone in the front pocket was not associated with various aspects of semen quality.

For other outcomes, little progress has been made in the period relevant for this report.

Human studies

Results concerning RF-EMF effects on the resting state EEG in the alpha frequency range continue to be inconsistent even in studies of high quality. Results published in 2021 showed either no effect (EEG study by Wallace et al. 2021a), an increase in alpha (EEG study by Dalecki et al, 2021), or a decrease in alpha power (MEG study by Wallace et al. 2021b). The search for reasons explaining the inconsistency indicate that results do not seem to depend on the definition of the alpha band frequency range, i.e. analyses for a fixed frequency range of 8-12 Hz yield the same results as analyses based on individual peak alpha frequency ranges. It seems that results vary with the eyes condition. While an EEG study found that the use of the eyes closed condition probably contributes to not observing RF-EMF effects, an MEG study observed that differences between exposure conditions seems to be more pronounced in the eyes closed condition. Since differences between exposure conditions are observed in both directions (increases and decreases) it is suggested not to use one-tailed statistical tests. Finally, in a parallel-group design study effects of RF-EMF exposure on functional connectivity and network properties have been reported. This study, however, indicated that SAR values do not seem to be the right metric to quantify neurophysiological effects. In MRI studies, however, it must be kept in mind that the method of assessment of exposure effects itself represents a complex EMF exposure situation during scanning. These exposure conditions are the same in the sham and the exposure condition related to the experimental signal.

Animal studies

As in previous years, there is again a variety of endpoints with diverging and inconclusive results. This year, most included studies show effects of exposure, a few do not. The exposure parameters, such as frequency, duration and exposure level, again vary considerably between studies. It is therefore difficult to draw general conclusions other than that under certain circumstances some effects from RF EMF exposure are observed in experimental animals. The observations of increased oxidative stress reported in previous SSM reports continue to be found, in contrast to effects on memory and behaviour.

Out of the 52 retrieved studies, 21 had to be excluded from analysis because of various reasons. It is of concern that 12 studies had to be excluded because of a flawed study design (mainly no sham-exposed group) or missing crucial information on dosimetry. Analyses that include all studies regardless of their quality will provide a biased picture.

In vitro studies

Eleven studies have been included in the report on the effect of RF exposure on mammalian cell cultures. They are related to several endpoints, such as brain development, proliferation, cell cycle, DNA damage, apoptosis, oxidative stress and gene and protein expression. Two of them also considered combined exposure to RF and chemical or physical agents.

Also this year, the published studies have been carried out by adopting different biological (cell type investigated, endpoint examined) and electromagnetic (frequency, modulation, SAR, exposure duration) experimental conditions. In addition, in some cases when a difference was recorded in exposed as compared to sham-exposed samples, it was reversible. Therefore, their biological relevance remains unclear.

Sammanfattning

Statiska fält

Epidemiologiska Studier

Sammanfattningen av epidemiologiska studier från förra året om exponering för statiska fält (eller MRI) och dess hälsoeffekter är i huvudsak oförändrad: Forskning om yrkesmässig MRI-exponering eller andra starka exponeringar från statiska fält är bristfällig och de underliggande mekanismerna för de samband som ibland observeras är oklara. Två nya studier publicerade 2021 utmanar inte den observationen. Med tanke på att exponeringen för statiska fält från MRI är relativt hög och antalet personer som är yrkesmässigt exponerade ökar är det berättigat med mer systematisk och omfattande forskning om detta ämne.

Studier på människa

Uppfattningströsklar för likström (DC), växelström (AC) och hybrid elektriska fält har systematiskt undersökts i en noggrant utformad studie på stor skala, som föregicks av en pilotstudie för att identifiera relevanta miljö- och experimentfaktorer. Studierna visade att uppfattningströsklarna är lägre under hybridexponeringsförhållanden än vid enbart DC eller AC-exponering. Denna forskning tillför värdefull information för en diskussion om acceptansen av sensorisk uppfattning av EF och hittills icke existerande referensnivåer och rekommendationer för hybrid exponering, vilket är viktigt för politiska beslutsprocesser kring konstruktionen av högspänningslikströms- och hybridkraftledningar.

Djurstudier

Starka magnetfält i intervallet 16,4 T påverkade motorisk koordination och balans hos råttor. Efter exponeringar för 2-12 T påverkades mineralinnehållet (Mg, Fe, Zn, Ca och Cu) i mössens huvudorgan, men inget konsekvent mönster observerades. Det är oklart om eller hur ett sådant fynd skulle kunna vara relevant för människor, till exempel när frivilliga exponeras för MRI i forskningsstudier.

Statiska magnetfält upp till 200 mT rapporterades orsaka vissa förändringar i glukosmetabolismen i hjärnan hos råttor och kunde leda till ångestliknande beteenden. När det gäller att adressera potentiella effekter på möss exponerade för statiska elektriska fält nära ultrahögspänningslikströmsledningar (±800 kV), visade studier inga långvariga hälsoeffekter, särskilt efter avslutad exponering.

Slutligen rapporterades det att malar konsekvent undviker statiska magnetfält större än 20 µT, och efter exponering på 110 mT hos kackerlacksnymfer uppstod en tillväxthämning.

Som tidigare år rapporterade alla studier vissa men inkonsekventa effekter. Detta kan bero på publiceringsbias. Flera forskargrupper använder sina specifika men liknande studiedesigner under åratal och rapporterar endast olika slutpunkter i sina efterföljande publikationer. Dessutom är de flesta studier inte hypotesdrivna.

Cellstudier

Under år 2021 publicerades ett stort antal in vitro-studier som behandlar effekten av statiska fält, men i de flesta fall var de relaterade till biomedicinska tillämpningar. Endast en studie inkluderades i rapporten som behandlar effekten av svaga statiska magnetfält på proliferation och oxidativ stress. Resultaten antyder att statiska fält kan inducera variationer i de biologiska slutpunkterna som övervägs, beroende på intensiteten (mellan 0,5 och 600 µT).

Lågfrekventa fält

Epidemiologiska studier

De nya studierna löser inte frågan om det konsekvent observerade sambandet mellan exponering för ELF-magnetfält (ELF-MF) och barncancer (leukemi) i epidemiologi är kausalt eller inte.

En ny studie som bedömer ELF-MF-exponering från inbyggda transformatorer indikerade en möjlig ökad risk för melanom och akut lymfatisk leukemi hos personer som hade bott i de högre exponerade lägenheterna under barndomen. Detta är en intressant observation som kräver uppföljning med tanke på det lilla antalet exponerade fall.

En nyligen genomförd uppföljning av en elektrikergrupp angående motorneuronsjukdom indikerade att möjligen nyare exponering för ELF-MF var mer relevant än tidigare exponeringar. Elektriska stötar utvärderades inte i denna studie. Frågan om magnetfält eller stötar är underliggande faktorer bakom de tidigare observerade sambanden med ALS/motorneuronsjukdom förblir obesvarad.

Studier på människa

Studier publicerade under den aktuella rapportperioden visade att låg relativ luftfuktighet (30%) förstärkte uppfattningen av AC-fält, och att samexponering av elektriska DC- och AC-fält ledde till en minskning av uppfattningsgränsen jämfört med antingen enbart AC eller DC. Överlag förstärktes uppfattningen av elektriska fält i närvaro av joner. Denna forskning tillför värdefull information för en diskussion om acceptansen av sensorisk uppfattning av EF och de hittills icke existerande referensnivåerna och rekommendationerna för hybrid exponering, vilket är viktigt för politiska beslutsprocesser kring konstruktionen av högspänningslikströms- och hybridkraftledningar.

En studie fann att stimulering av nackloben oberoende av retinal aktivering kan påverka uppfattningen av fosfener, och en annan studie observerade viss evidens för en aktivering av den parasympatiska aktiviteten efter en 15 minuters exponering för lågfrekventa magnetfält med frekvensen 50 Hz.

Djurstudier

I kontrast till tidigare rapporter av rådet identifierades endast en studie med gnagare. Hyperglykemi hos matade eller fastande råttor rapporterades efter en enda 15 minuters lång exponering för ELF-MF vid 60 Hz och 3,8 mT. Tre ytterligare studier på honungsbin som behandlade ultra-högspänningsöverföringsteknik och använde ELF-EF (50 Hz, 5 - 34,5 kV/m) bekräftade att hemolymfa, antioxidantssystem och beteende hos honungsbin påverkas när man applicerar 50 Hz EF så lågt som 5,0 eller 11,5 kV/m. Hos fruktflugor som exponerades för 75 Hz 500 µT ELF-MF ökade reaktiva syreföreningar, medan rörelse, kläckningshastighet och livslängd minskade. Efter 50 Hz-exponeringar upp till 1 T hos myggslarver upptäcktes allvarliga missbildningar. Slutligen orsakade 20 Hz och 50 Hz ELF-MF små men signifikanta förändringar i vingslagfrekvensen hos gräshoppor, och gräshoppor anpassade sig till den exakta frekvensen hos det applicerade ELF-MF.

Sammanfattningsvis rapporterades olika effekter återigen efter ELF-MF-exponering i olika djurmodeller, främst icke-mammaliana. Förutom oxidativ stress är rimliga mekanismer fortfarande oklara.

Cellstudier

In vitro-studier publicerade under år 2021 utvärderade effekten av ELF-fält på cellproliferation, DNA-integritet, ROS-produktion och cirkadisk rytm. Alla beskrivna effekter verkar bero på experimentella förutsättningar (celltyper, exponeringstid, frekvens, fältintensitet) och de inkonsekventa mönstren innebär att resultaten är svåra att tolka.

Intermediära fält

Epidemiologiska studier

En enda studie om IF-MF-exponering och graviditetsutfall publicerades under den relevanta rapportperioden; studien tillåter inte fasta slutsatser angående möjliga hälsoeffekter från exponering för IF-MF.

Studier på människa

Som för tidigare rapportperioder fanns det ingen mänsklig experimentell studie inom det intermediära frekvensintervallet.

Djurstudier

Inom området 20 - 100 kHz resulterade totalt sett två mösstudier inte i negativa effekter på beteende och genotoxikologi.

Cellstudier

Endast en artikel har inkluderats i rapporten, som handlar om exponering av svinäggceller för att utvärdera mognad och embryonal utveckling. Författarna rapporterar att exponering för lägre - men inte för högre - intensitet IF-EMF kan störa äggmognad.

Radiofrekventa fält

Epidemiologiska studier

Flera nya epidemiologiska studier fann ingen association mellan mobiltelefonanvändning och hjärntumörer. Den internationella MOBI-kids fallkontrollstudien är hittills den största studien om detta ämne. Övergripande fann studien inget bevis för ökade risker. Faktum är att en trend med minskad risk vid ökande mobiltelefonanvändning observerades. Dock tyder detaljerade analyser på att denna observation sannolikt är snedvriden på grund av proxyintervjuer och prodromala symtom. Detta illustrerar att fallkontrollstudier kan ha begränsad potential att upptäcka små risker eftersom de är benägna för olika typer av bias.

Ett kompletterande tillvägagångssätt är att analysera tidsmässiga trender i förekomsten av hjärntumörer och jämföra med ökningar i mobiltelefonabonnemang. Flera sådana artiklar har publicerats om olika typer av tumörer i huvudområdet, som inte indikerar en ökning hos vuxna i linje med ökningen av mobiltelefonanvändning.

Ett antal systematiska översikter postulerar en association mellan mobiltelefonanvändning och spermakvalitet. Dock var dessa översikter till stor del baserade på studier av låg kvalitet som var sårbara för selektionsbias och som ofta använde sig av grova och retrospektiva exponeringsbedömningsmetoder.

En artikel som utvärderade data från två stora prospektiva kohortstudier fann liten evidens för att bära en mobiltelefon i en främre byxficka påverkade manlig fertilitet, även om en signifikant effekt hos män med en BMI på mindre än 25 kg/m² förtjänar uppföljande undersökning. I samma studie var att bära en telefon i fickan inte förknippad med olika aspekter av spermakvalitet.

För andra utfall har lite framsteg gjorts under den relevanta perioden för denna rapport.

Studier på människa

Resultaten angående RF-EMF-effekter på vilande EEG i alfafrekvensområdet fortsätter att vara inkonsekventa även i studier av hög kvalitet. Resultat publicerade 2021 visade antingen ingen effekt (EEG-studie av Wallace et al. 2021a), en ökning av alfa (EEG-studie av Dalecki et al, 2021), eller en minskning av alfakraften (MEG-studie av Wallace et al. 2021b). Sökandet efter anledningar som förklarar inkonsekvensen indikerar att resultaten inte verkar bero på definitionen av alfabandsfrekvensområdet, dvs analyser för ett fast frekvensområde på 8-12 Hz ger samma resultat som analyser baserade på individuella alfatoppfrekvensområden. Det verkar som att resultaten varierar med ögonens tillstånd. Medan en EEG-studie fann att användningen av ”ögonen stängda”-tillståndet förmodligen bidrar till att inte observera RF-EMF-effekter, observerade en MEG-studie att skillnaderna mellan exponeringsförhållanden verkar vara mer uttalade i ”ögonen stängda”-tillståndet. Eftersom skillnader mellan exponeringsförhållanden observeras åt båda hållen (ökningar och minskningar) föreslås det att inte använda ensidiga statistiska tester. Slutligen har en studie i parallellgruppsdesign rapporterat effekter av RF-EMF-exponering på funktionell anslutning och nätverksegenskaper. Denna studie indikerade dock att SAR-värden inte verkar vara den rätta metriken för att kvantifiera neurofysiologiska effekter. I MRI-studier måste emellertid metoden för bedömning av exponeringseffekter själv representera en komplex EMF-exponeringssituation under skanning. Dessa exponeringsförhållanden är desamma i både sham- och exponeringsförhållandet relaterat till den experimentella signalen.

Djurstudier

Som tidigare år finns det en mängd olika ändpunkter med divergerande och otillräckliga resultat. I år visar de flesta inkluderade studier effekter av exponering, några gör det inte. Exponeringsparametrarna, såsom frekvens, varaktighet och exponeringsnivå, varierar återigen betydligt mellan studier. Det är därför svårt att dra allmänna slutsatser annat än att under vissa omständigheter observeras vissa effekter av RF EMF-exponering på försöksdjur. Observationerna av ökad oxidativ stress som rapporterats i tidigare SSM-rapporter fortsätter att påträffas, i motsats till effekter på minne och beteende.

Av de 52 hämtade studierna måste 21 uteslutas från analysen av olika skäl. Det är oroande att 12 studier måste uteslutas på grund av en bristfällig studiedesign (främst ingen sham-exponerad grupp) eller bristande väsentlig information om dosimetri. Analyser som inkluderar alla studier oavsett deras kvalitet kommer att ge en snedvriden bild.

Cellstudier

Elva studier har inkluderats i rapporten om effekten av RF-exponering på mammaliska cellkulturer. De är relaterade till flera ändpunkter, såsom hjärnutveckling, proliferation, celldelning, DNA-skada, apoptos, oxidativ stress samt gen- och proteinuttryck. Två av dem övervägde även kombinerad exponering för RF och kemiska eller fysiska agenter. Även detta år har de publicerade studierna genomförts genom att anta olika biologiska (undersökta celltyper, undersökt slutpunkt) och elektromagnetiska (frekvens, modulation, SAR, exponeringstid) experimentella förhållanden. Dessutom, i vissa fall när en skillnad registrerades i exponerade jämfört med sham-exponerade prover, var den reversibel. Därför förblir deras biologiska relevans oklar.

Preamble

In this preamble we explain the principles and methods that the Council uses to achieve its goals. Relevant research for electromagnetic fields (EMF) health risk assessment can be divided into broad sectors such as epidemiologic studies, experimental studies in humans, animals and in vitro studies. Where relevant, studies on biophysical mechanisms, dosimetry, and exposure assessment can also be considered. A health risk assessment evaluates the evidence within each of these sectors with the aim to eventually weigh together the evidence across the sectors to provide a combined assessment. Such a combined assessment should address the question of whether or not a hazard exists, i.e. if a causal relation exists between exposure and some adverse health effect. The answer to this question is not necessarily a definitive yes or no, but may express the likelihood for the existence of a hazard. If such a hazard is judged to be present, the risk assessment should also address the magnitude of the effect and the shape of the exposure-response function, i.e. the magnitude of the risk for various exposure levels and exposure patterns.

As a general rule, only articles that are published in English language peer-reviewed scientific journals[1] since the previous report are considered by the Council. A main task is to evaluate and assess these articles and the scientific weight that is to be given to each of them. However, some of the studies are not included in the Council report either because the scope is not relevant (e.g. therapeutical studies), or because their scientific quality is insufficient. For example, poorly described exposures and missing unexposed (sham) controls are reasons for exclusion. Such studies are normally not commented upon in the annual Council reports (and not included in the reference list of the report)[2]. Reasons why individual studies were excluded are listed in the appendix to the report. Systematic reviews and meta-analyses are mentioned and evaluated, whereas narrative and opinion reviews are generally not considered.

The Council considers it to be of importance to evaluate both studies indicating that exposure to electromagnetic fields has an effect as well as studies indicating a lack of an effect. In the case of studies indicating effects, the evaluation focuses on alternative factors that may explain the result. For instance, in epidemiological studies it is assessed with what degree of certainty it can be ruled out that an observed effect is the result of bias, e.g. confounding or selection bias, or chance. In the case of studies that do not indicate effects, it is assessed whether this might be the result of (masking) bias, e.g. because of too small exposure contrasts or too crude exposure assessment. It also has to be evaluated whether the lack of an observed effect could be the result of chance, a possibility that is a particular problem in small studies with low statistical power. Obviously, the presence or absence of statistical significance is only one of many factors in this evaluation. Indeed, the evaluation considers a number of characteristics of the study. Some of these characteristics are rather general, such as study size, assessment of participation rate, level of exposure, and quality of exposure assessment. Particularly important aspects are the observed strength of the association and the internal and external consistency of the results including aspects such as exposure-response relation. Other characteristics are specific to the study in question and may involve aspects such as dosimetry, method for

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[1] Articles are primarily identified through searches in relevant scientific literature data bases; however, the searches will never give a complete list of published articles. Neither will the list of articles that do not fulfil quality criteria be complete.

[2] Articles not taken into account due to insufficient scientific quality are listed in an appendix and reasons for not being taken into account are indicated.

assessment of biological or health endpoint(s) and the relevance of any experimental biological model used.[3]

It should be noted that the result of this process is not an assessment that a specific study is unequivocally negative or positive or whether it is accepted or rejected. Rather, the assessment will result in a weight that is given to the findings of a study. The evaluation of the individual studies within a sector of research is followed by the assessment of the overall strength of evidence from that sector with respect to a given outcome. This includes taking into account the observed magnitude of the effect and the quality of the studies.

In some cases, in an overall evaluation phase, the available evidence may be integrated over the various sectors of research. This involves combining the existing relevant evidence on a particular endpoint from studies in humans, from animal models, from in *vitro studies*, and from other relevant areas. In such a final integrative stage of evaluation the plausibility of the observed or hypothetical mechanism(s) of action and the evidence for that mechanism(s) have to be considered. The overall result of the integrative phase of evaluation, combining the degree of evidence from across epidemiology, human and animal experimental studies, *in vitro* studies and other data depends on how much weight is given on each line of evidence from different categories. For assessing effects on humans, human epidemiology is, by definition, an essential and primordial source of evidence since it deals with real-life exposures under realistic conditions in the species of interest. The epidemiological data are, therefore, given higher weight in the overall evaluation stage. However, epidemiological data has to be supported by experimental studies to establish a causal link between exposure and health. Where this is relevant and possible, also effects on other species are taken into account.

An example demonstrating some of the difficulties in making an overall assessment is the evaluation of ELF magnetic fields and their possible causal association with childhood leukaemia. It is widely agreed that epidemiology consistently demonstrates an association between exposure to ELF magnetic fields and an increased occurrence of childhood leukaemia. However, there is lack of support for a causal relation from observations in experimental models and a plausible biophysical mechanism of action is missing. This had led the International Agency for Research on Cancer (IARC) to the overall evaluation of ELF magnetic fields as “possibly carcinogenic to humans” (Group 2B).

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[3] For a further discussion of aspects of study quality, see for example the Preamble of the IARC (International Agency for Research on Cancer) Monograph Series (IARC, 2002).

1 Static fields

1.1 Epidemiological studies

Last years' report summarised that occupational exposure from magnetic resonance imaging (MRI) caused acute and transient symptoms, but that long-term consequences for health remained unclear. Research on occupational MRI exposure was scarce and underlying mechanisms for occasionally observed associations were unclear, this included also a new study on sleep problems in persons occupationally exposed to MRI.

Tracy et al. [1] analysed existing data from the Normative Aging Cohort study, a study that had been set up in 1963, when participants were on average 42 years old (range 21-81) in relation to natural geomagnetic field exposure from the sun. Participants were free of chronic conditions at baseline and attended physical examinations every 3-5 years. Data from 2000-2013 were analysed in this publication (average age 75.8 years). The authors evaluated associations between solar geomagnetic activity (solar magnetic field (interplanetary magnetic field, AKA heliospheric magnetic field), sunspot number (SSN), and geomagnetic activity (Kp Index) and total white blood cell (WBC), neutrophil, monocyte, lymphocyte, eosinophil, and basophil concentrations. 728 participants with 2048 observations were included into the analysis. Exposure windows of the day of the measurements and moving averages over one month were calculated. For the interplanetary magnetic field exposure, levels ranged from 0 to 28 nT. Mixed effects linear regression models with random intercepts per participant were used to account for the repeated measurements per participant; models were adjusted for a range of potential confounders. The authors reported associations, especially of interplanetary magnetic field and reductions in neutrophil and basophil counts with longer exposure windows. Several potential pathways are discussed by the authors, including effects on melatonin or the circadian rhythm.

Strength of the study is the relatively large data base with independently collected exposure data and a range of potential confounders that were taken into account, including for example air pollutants or alcohol consumption and smoking. The authors do not discuss the clinical relevance of the findings.

Bravo et al. [2] performed a survey among medical doctors, research staff and healthcare workers from different hospital radiology and research units across Italy. Of 240 workers included, 177 (74%) reported having accessed the controlled access room of an MRI hospital scanner within the past year at least once. These 74% were subsequently categorized as "exposed". Eleven "core" symptoms that have been previously reported to occur when in the vicinity of an MRI scanner were evaluated, including vertigo, nausea, tinnitus, metallic taste, magnetophosphenes, headache, drowsiness, concentration problems, balance instability, memory loss, and sleep disorders. The frequency of the symptoms was estimated based on a Likert scale (never or less than once a month, at least once a month, one to four times per week, more than four times per week). No differences were observed with occurrence or frequency of symptoms between exposed and unexposed workers. Sensitivity analysis among persons working with MRI ≤ 1.5T or ≥ 3T or evaluating the number of annual MRI procedures confirmed this pattern. Higher job stress was associated with higher symptom reports, independently of exposure. The authors conclude that they did not observe associations between occupational exposure to static fields from MRI and symptoms but found that stress played an important role.

Compared to previous studies of workers exposed to strong static fields from MRI scanners, the sample size is relatively large, although still limited in terms of statistical power. Weakness of the study is that exposure was very vaguely defined and included personnel that may not enter the scanning room at all, but just stay within the access-restricted area only. Comparisons with previous publications is somewhat hampered, as earlier studies tried to either assess exposure (Schaap et al 2013, de Vocht et al 2015), or tried to assess access to MRI scanner rooms during procedures in a more detailed way. Likely, the occupational group categorised as “exposed” in this study includes many unexposed or infrequently exposed individuals. The observation that stress contributes to symptom reporting has been made before and is a factor that should be addressed in future studies.

Last years’ summary on static field (or MRI) exposure and health effects is essentially unchanged: Research on occupational MRI exposure or other strong static field exposures is scarce and underlying mechanisms for occasionally observed associations are unclear. The two new studies do not challenge that observation. Given that exposure to static fields from MRI is relatively high, and the number of occupationally exposed persons is increasing, more systematic and comprehensive research on this topic is warranted.

1.2 Human Studies

In the reporting period two studies from the same group, a pilot study (Jankowiak et al. [3]) and the main study (Kursawe et al. [4]) were published, which investigated systematically perception thresholds among others for direct current electric fields (DC).

In the face of a changing energy supply, high voltage power lines are becoming increasingly important for the transport of energy. In a pre-study to the main study by Kursawe et al., Jankowiak et al. performed a study, which included 11 participants (9 males and 2 females, age range: 23 to 33 years, mean ± SD: 25.45 ± 3.17 years). The pre-study investigated the impact of various environmental (humidity) and experimental factors (ramp slope, exposure duration, air ions and polarity), which potentially could influence perception threshold of EF. Using the same highly specialized whole-body exposure laboratory as in the main study and the same assessment methodology they investigated the effect of a direct current electric field (DC) exposure of 16, 20, 30, and 44 kV/m and an alternating current (AC) exposure of 10, 20, and 30 kVm on different test days. The results were used to identify the setting for the large-scale study (Kursawe et al.). They identified relative humidity as an environmental factor influencing the perception of AC and DC electric fields in different ways and determined an appropriate ramp slope and exposure duration for future studies. They observed that perception threshold was lower under hybrid EF exposure than under DC EF or AC EF exposure alone and suggested that an EF strength of 4 kV/m AC and a 6-30 kV/m DC should be applied in large-scale studies.

The main study by Kursawe et al. aimed at determining human detection thresholds for DC fields. They also investigated detection thresholds for AC and hybrid electric fields (various DC, constant AC). In total 203 subjects (20 to 79 years, 103 males and 100 females; almost equally distributed across age ranges: 20-34 years: 51, 35-49 years: 49; 50-64 years: 53, 65-79 years: 50) were exposed to DC, AC and hybrid electric fields (EFs) under high safety standards. Subjects with electronic implants, not removable piercings, self-reported electromagnetic hypersensitivity, dermatosis, neurological disorders and claustrophobia were not included. The design of the experiments was double-blind, possible auditory cues were masked by a 65.8 dB (A) white noise. To measure the participant’s ability to perceive EF and to calculate detection thresholds two methods were used. One was the signal detection theory

(SDT), which requires subjects to distinguish signal trials where EF was present from sham trials with no exposure (yes/no answer). The outcome parameter in this approach is d', which is based on hits and false alarms. The other measure was a staircase procedure following the single interval adjustment matrix (SIAM). This measure follows an adaptive algorithm based on participant's response to a given trial. The SIAM procedure is described as follows: "*Starting with a predefined EF, the strength was reduced by 4 kV/m when the participant's response was correctly "yes" or increased by 4 kV/m when the participant's response was "no" referring to step size. After the false alarm, EF was increased by 8 kV/m. Correct responses to sham trials did not entail a change in EF strength. Reversals are defined as the point where the participant's response leads to a direction change of the increasing or decreasing EF strength. After five reversals, the step size was reduced to 2 kV/m for a more finegrained resolution of the last three reversals. The average of these three EF strengths was defined as an individual detection threshold that refers to the field strength where EF is just perceptible*" (Kursawe et al., pages 4 and 5 of 14).

During the experimental tests, subjects wore long trousers while the forearms were left uncovered. The temperature was set to 25 °C and the humidity to 50%. For every EF type (DC: 4 – 44 kV/m, DC with ion currents: 80, 200, 300, 400 nA/m^2, AC: 4 – 30 kV/m, hybrid: AC 4 kV/m and DC – 44 kV/m and hybrid: AC 4 kV/m and DC: 18 and 24 kV/m with ion currents: 10 nA/m^2), subjects performed two SDT trials. In two subgroups, half of the SDT sessions were performed under 30% relative humidity (n=24) or 70% relative humidity (n=25). For DC a repeated measures ANOVA with EF strength (14, 22, 30, and 38 kV/m) and ion presence (yes, no) revealed a statistically significant main effect of EF strength as well as a significant interaction between both factors. Sensitivity increased with field strength and was higher when ions were present (except for the 14 kV/m DC exposure situation). For AC a repeated measures ANOVA with the factor field strength (8, 16, 24, and 30 kV/m) also showed a significant field strength effect. The detection thresholds of hybrid EF were lower than those of DC or AC fields alone. The SDT detection thresholds were 18.69 (SD 8.42) kV/m for DC, 18.22 (SD 5.65) kV/m for DC with ions, 14.16 (SD 7.96) for AC and 6.76 (SD 6.26) for hybrid exposure. The detection thresholds assessed by SIAM were 23.17 (SD 8.98) kV/m for DC, 17.57 (SD 7.55) kV/m for AC and 14.34 (SD 6.29) for hybrid exposure. EF perception was enhanced in the presence of ion current exposure. DC EF perception was facilitated by a high humidity (70%) whereas low relative humidity strengthened the perception of AC fields.

The authors conclude that "*although the average detection thresholds do not undercut the existing reference levels for DC and AC EFs, the study found evidence for successful EF perception around these reference levels in a small subset of participants*" (Kursawe et al. page 13 of 14). Since perception of EF is not yet an adverse health effect the results of this study do not question existing reference values for AC and DC exposure. However, the findings might stimulate a discussion on the acceptability of sensory perception in the context of EF exposure. Furthermore, the results can be used in the discussion of not yet existing reference values and recommendation for hybrid exposure.

Detection thresholds for direct current (DC), alternating current (AC), and hybrid electric fields have been systematically investigated in a carefully designed large scale study, which was preceded by a pilot study to identify relevant environmental and experimental factors. The studies revealed that perception thresholds are lower in hybrid exposure conditions than in DC or AC exposure alone and that that the perception of DC electric fields was facilitated by high humidity (70%). This research adds valuable information for a discussion of the acceptability of sensory perception of EF and the so far not existing reference levels and recommendations for hybrid exposure, which is important for political decision-making processes around the construction of high voltage DC power and hybrid power lines.

Table 1.2.1: Human studies on exposure to static (and low frequency electric fields - EF)

Endpoints	Reference	Exposure condition	Sample	Results
Perception threshold of AC and DC EF	Jankowiak et al.	DC: 10, 16, 20, 30 44 kV/m; AC: 10, 20, 30 kV/m in the presence of varying experimental (ramp slope, exposure duration, air ions and polarity) and environmental conditions (humidity)	11 healthy subjects (9 males, 2 females), age range: 23 to 33 years, mean ± SD: 25.45 ± 3.17 years	EF strength of 4 kV/m AC and 6-30 kV/m DC should be applied in large-scale studies
	Kursawe et al.	DC: 4 – 44 kV/m, AC: 4 – 30 kV/m, hybrid: DC 4 – 44kV/m, AC: 4 kV/m with and without ions at different humidities (30%, 50% and 70%)	203 healthy subjects (103 males, 100 females), Age range 20-79 years	SDT detection thresholds[1)]: DC 18.69 (SD 8.42) kV/m DC with ions 18.22 (SD 5.65) kV/m, AC: 14.16 (SD 7.96) kV/m Hybrid exposure: 6.76 (SD 6.26) kV/m SIAM detection thresholds [2)] : DC: 23.17 (SD 8.98) kV/m, AC, 17.57 (SD 7.55) kV/m hybrid exposure: 14.34 (SD 6.29) kV/m

[1)] SDT: Signal detection theory, [2)] SIAM: Single interval adjustment matrix

1.3 Animal studies

For the reporting year 2021, six experimental studies using rats and mice were identified, while two papers described exposures of non-mammalian species.

In one study rats were exposed to high static fields of 10.5 T or 16.4 T, whereas in mice high static field strengths between 2 and 12 T were tested. The effects of 200 mT SMF exposure on glucose metabolism in rats' brain and behaviour were evaluated in another study. Furthermore, three studies in mice were run with SEF in the range of 9.2 to 56.3 kV/m.

Studying swimming behaviour, relative growth rate and brain proteins, the non-mammalians catfish and cockroach were used.

1.3.1 Brain and behaviour

Shuo et al. [5] exposed male Wistar rats for 1 h/day on 15 consecutive days to 50, 100, and 200 mT SMF. The fourth group was sham exposed. After 1, 5, and 15 days of SMF exposure glucose metabolism in rats' brain (n=3 per group) was evaluated by micro-positron emission tomography (μ-PET). Histopathology of the hippocampus and Western blots of the two rate-limiting enzymes hexokinase 1 (HK1) and 6-phosphate fructokinase-1 (PFK1) were done following 1, 5, 10, 15 days of exposure (n=5 per group). Finally, behaviour was tested in sham and 200 mT-exposed rats (n=10 per group) after 1, 5, 10, 15 days. µ-PET results showed SMF intensity-dependent increased glucose metabolism after 1 day, "weakened" metabolism after 5 days, and after 15 days similar glucose metabolism to that before exposure. After 5 days a SMF strength-related and significant decrease in expression of HK1 and PFK1 in brain started. After 15 days of exposure (but not after 1, 5, 10 days) pyknosis, oedema of neurons, and slight widening of the perivascular space was observed in cortex, hippocampus and striatum. The Open Field Test demonstrated that the total distance, surrounding distance, activity time, and climbing and standing times significantly decreased after 10 and 15 days of 200 mT SMF exposure. Summarizing, the applied SMF may change glucose metabolism in the brain and could result in anxiety-like behaviours. The pathological and behavioural changes occurred later than those reported for glucose metabolism.

Tkač et al. [6] tested in C57BL/6 mice (n = 12 males + 12 females per sham and exposure group) potential neurochemical, behavioral or cognitive effects following 4- or 8-week exposures (3 h/d, 2 x/wk) to ultra-high SMF of 10.5 T or 16.4 T. In vivo ^{1}H MRS (magnetic resonance spectroscopy) data did not demonstrate changes in hippocampal neurochemical profile. Morris water maze (MWM) test did not reveal learning and memory deficits in exposed mice, and the fear conditioning test did not result in significant differences among groups. Also, a locomotor deficit or impaired motor coordination was not observed in the acceleration rotarod. But the balance-beam–walking test demonstrated SMF-induced changes in motor coordination and balance in mice exposed to 16.4 T. In addition, a tight-circling locomotor behavior during MWM tests was found 1-4 days after the last SMF exposure at 16.4 T but not at 10.5 T. The authors conclude that exposure to 16.4 T may lead to long-term impairment of the vestibular system in mice, and the findings would have "serious implications for the safety of subject participation in MRI/MRS research studies at ultra-high magnetic fields".

1.3.2 Physiology, pathophysiology and oxidative stress

In continuation of their research Wang et al. [7] continously exposed groups of 12 male C57BL/6 mice at 3 different high static magnetic field (HiSMF) strengths (2–4 T, 6–8 T, and 10–12 T) for 28 days (compare Wang et al. [8], Swedish Radiation Safety Authority [9]). A fourth group of 12 mice was sham-exposed. In the major organs (liver brain, kidney and heart) the levels of Mg, Fe, Zn, Ca, and Cu were measured by atomic absorption spectroscopy. Compared to sham control 2-4 T HiSMF increased Zn in brain, Mg, Fe, and Ca in kidneys. Following 4-wk exposure at 6-8 T, in the liver Zn, in the heart Fe, and again in kidneys Mg, Fe, and Ca were increased; but in the heart Zn and Ca was decreased. Finally, the 10–12 T HiSMF exposure significantly increased Mg in the kidneys, Fe in liver and kidneys, and Cu in brain, whereas Zn in the kidneys, Ca in brain and heart were decreased. Overall, HiSMF altered levels of Mg, Fe, Zn, Ca, and Cu content in the main organs of mice. Regardless of the authors' discussion that different magnetic properties and various ion channels may cause the different responses to SMFs, no consistent pattern is visible. Thus, verification of the results in an independent further study would be recommended.

Yu et al. [10] explored potential effects in the hippocampus of mice. In an open-air environment close to ultra-high-voltage direct-current (UHVDC) transmission lines (±800 kV) eight 5-wk old male ICR mice were continuously exposed to static electric field (SEF) strengths of 9.20 - 21.85 kV/m for 35 days. At another site with the same environmental conditions 8 males were sham-exposed. Mice' body weight was measured weekly. Following

35 days of exposure, hippocampal Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and calcineurin (CaN) expression levels were detected. In different subgroups morphology (n=5) and ultrastructure (n=3) were observed by light microscopy and transmission electron microscopy (TEM), respectively. Body weight development, CaMKII and CaN expression levels, and hippocampal histomorphology did not differ between exposed and sham-exposed mice. But cytoplasmic vacuolization of the hippocampal neurons was observed following SEF exposure. The authors conclude that hippocampal neuron ultrastructure damage may cause SEF-exposure-induced memory decline in mice which they reported already in 2016 (Xu et al. 2016). However, according to experimental data of the same research group this holds only true for 7-d short-term SEF exposures (35 kV/m or 56.3 kV/m), but not for longer exposure durations up to 49 days (Xu et al. 2018; Di et al. 2019).

Under laboratory conditions, Di et al. [11] continuously exposed 34 male 4-week-old ICR mice to a SEF of 56.3 kV/m up to 28 days. A further 34 males were sham-exposed. Testosterone levels and indicators of testicular oxidative stress (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA)) were studied in 10 mice each following 3 days and 28 days of exposure, and after a recovery phase without exposure of further 7 days, respectively. On day 28 effects on testosterone synthesis were determined by mRNA expression and protein levels of steroidogenesis acute regulatory (StAR), peripheral-type benzodiazepine receptor (PBR) and CYP11A1 (which encodes the P450scc enzyme that catalyzes the first and rate-limiting step of steroid biosynthesis). Finally, in 4 mice per group the ultrastructure of Leydig cells was investigated by use of TEM. Serum testosterone and testicular oxidative stress levels were not affected after 3 days and after the recovery phase. But following 28 days of SEF exposure, serum testosterone levels, testicular GSH-Px activity, the mRNA and protein levels of testicular StAR, PBR, CYP11A1 decreased significantly, and testicular MDA increased significantly. TEM showed damaged mitochondrial structure in Leydig cells, which (according to the authors) could reduce cholesterol (which is a necessary precursor of testosterone synthesis) transport from cytoplasm to mitochondria resulting in an inhibition of testosterone synthesis. Using the same exposures and a very similar experimental design, Dong et al. [12] studied the effects of SEF on the spleen of male 4-wk old ICR mice. 34 mice were continuously exposed to 56.3 kV/m up to 21 days. 34 further mice were sham-exposed. The oxidative stress indices (SOD, GSH-Px, calcineurin, nitric oxide synthase, MDA) and the mRNA expression levels of tumour necrosis factor-α (TNF-α) and nuclear factor kappa B (NF-κB) in the spleens were determined in groups of 10 mice each after 7, 14, and 21 days, respectively. After 21 days in 4 further mice per group the ultrastructure of the spleens was investigated by TEM. SEF exposure of 21 days significantly increased the oxidative stress levels, but not after 7 and 14 days. Also after 21 days TNF-α and NF-κB were increased, and in splenic lymphocytes a separation of nucleus and nuclear membrane, the disappearance of mitochondrial membrane, and the deficiency of mitochondrial cristae was observed. The authors recommend to test in a further study whether after an exposure stop the levels of oxidative stress and of TNF-α and NF-κB (indicating spleen inflammation), and damaged splenic lymphocytes recover to normal. However, the same researchers (Dong et al. [13]) included already a recovery group in their previous study described above. Furthermore, and given to their large experience with testing of SEF effects in mice (Xu et al. [14], Wu et al. [15], Di et al. [16], Lin et al. [17], Xu et al. [14], Di et al. [18], Di et al. [19]) the authors should have justified the selected 56.3 kV/m and duration of exposure.

1.3.3 Studies in Non-Mammalians

Hunt et al. [20] determined the swimming direction of glass catfish after magnetic stimulation. The same 13 fish were in a radial Y-maze SMF- or sham-exposed for 2 weeks.

All 13 fish were tested in constant location and 12 in an alternating (magnet's) location experiment. A Permanent Neodymium Rare Earth Magnet (with a horizontal magnetic flux of 577 mT at the magnet's surface) and a sham magnet were placed 10 cm from the end of the Y-maze arm. The MF strengths inside the maze was calculated by COMSOL (which is a multiphysics simulation software). Each trial (constant location or alternating location of magnet in the Y-maze arms) was recorded for 30 minutes by overhead cameras and was repeated four times for each condition for a total of 24 trials. The results indicate, according to the authors, that glass catfish consistently swim away from magnetic fields greater than 20 µT and show adaptability to changing magnetic field direction and location.

Ilijin et al. [21] described in a third paper effects in cockroach nymphs following chronic exposure to SMF as well as to ELF-MF (compare Todorovic et al. [22, 23], Swedish Radiation Safety Authority [24, 25]). Again, one month-old cockroach nymphs (n=20-25 per group) were exposed for 5 months each to (1) 110 mT SMF, (2) 50 Hz, 10 mT ELF-MF (see chapter 2.3.4), (3) sham exposure. The exposure effects on the insects' relative growth rate (RGR), in brain tissue acetylcholinesterase (AChE) and (HSP70) were investigated.

Compared to controls, insects' relative growth rate and brain levels of heat shock protein 70 were significantly increased, whereas AChE activity significantly decreased.

1.3.4 Summary and conclusions on static magnetic and electric field animal studies

Strong MF in the range of 16.4 T affected motor coordination and balance in rats. Following exposures to 2 -12 T the mineral element content (Mg, Fe, Zn, Ca, and Cu) in mice' main organs was inconsistently affected.

It is unclear if or how such a finding would also translate into relevance for humans, for example when volunteers are exposed to MRI in research studies.

SMF of up to 200 mT can alter glucose metabolism in the brain of rats and result in anxiety-like behaviours.

Addressing potential effects on mice exposed to SEF close to ultra-high- voltage direct-current transmission lines (±800 kV), mouse studies did not show effects on body weight development and hippocampal enzymes and histomorphology. However, serum testosterone and testicular oxidative stress levels decreased but recovered after exposure stop. Whether after an exposure stop the increased levels of splenic oxidative stress and inflammation marker, and of damaged lymphocytes in the spleen recover to normal was not tested.

Finally, it was reported that catfish consistently avoid static magnetic fields greater than 20 µT, and after 110 mT exposure in cockroach nymphs a growth retardation occurred.

As in previous years, all studies reported some but inconsistent effects. This may be due to publication bias. Several research groups use their specific but similar study design over years and report only different endpoints in their successive publications. In addition, most studies are not hypothesis-driven.

Table 1.3.1: Animal studies on exposure to static fields

Endpoints	Reference	Exposure condition	Species & Strain	Results
Rodent studies				
Brain and behaviour	Shuo et al. (2021)	50, 100, 200 mT 1 h/d 1, 5, 10, 15 d	Wistar rat	Glucose metabolism in brain affected. Anxiety-like behaviours (200 mT)
	Tkač et al. (2021)	10.5, 16.4 T 3 h/d, 2 x/wk 4 and 8 wk	C57BL/6 mice	Tight circling behaviour, impaired motor coordination and balance at 16.4 T
(Patho)Physiology	Yu et al. (2021)	9.20 – 21.85 kV/m (Open air environment) 35 d	ICR mice	Vacuolization of hippocampal neurons
	Di et al. (2021)	56.3 kV/m 3 d, 28 d, 28 d + 7d recovery	ICR mice	After 28 d inhibition of testosterone synthesis, which recovered after 7 d
	Dong et al. (2022)	56.3 kV/m 7, 14, 21 d	ICR mice	After 21 d increased oxidative stress in spleen and damaged splenic lymphocytes
	Wang et al. (2021)	2-4, 6-8, 10-12 T 28 d continuously	C57BL/6 mice	altered levels of Mg, Fe, Zn, Ca, and Cu content in mice' main organs
Studies in Non-Mammalians				
Locomotion and behaviour	Hunt et al. (2021)	> 20 µT 30 min trials	Glass catfish	Catfish consistently swim away from SMF >20 µT
(Patho)Physiology	Ilijin et al. (2021)	110 mT 5 mo	Cockroach nymphs of *Blaptica dubia*	RGR, brain HSP 70 increased, brain AChE decreased

Abbreviations: AChE: acetylcholinesterase; d: day(s); h: hours; mo: month(s); RGR: relative growth rate; SEF: static electric field; SMF: static magnetic field; wk: week(s)

1.4 Cell Studies

In the year 2021 one study only was included in the report, dealing with the effect of weak static magnetic fields on oxidative stress and cell growth.

HT-1080 human fibrosarcoma cells were exposed to weak static magnetic fields (SMFs) for four consecutive days from 0.5 to 600 µT (Gurhan et al [26]). Control cells were held at 45 µT considered as the nominal earth′s MF. The data were processed in blinded fashion and the temperature was continuously monitored during the experiments. The cell growth rates were increased between 200 and 400 μT (n=12 for each group) and decreased at 0.5 and 600 μT (n=12) and varied with the angle of the SMFs. Concentrations of mitochondrial calcium (n =12 for each group) and membrane potential (n=12 for each group) measured by fluorescence dyes have shown an increase linked to the magnitude of the SMFs while decreases in intracellular pH (n =12 for each group) were observed. The reactive oxygen species H_2O_2 were increased at 100 and 200 μT, decreased at 300 μT and increased again at 500 and 600 μT (n=63 for each group). Nitric Oxide (NO) was decreased with respect to the control for 0.5 μT, 100, 400, and 600 μT while mitochondrial superoxide production was decreased with all SMF values (n=63 for each group). These results indicate that SMFs could accelerate and inhibit cell growth rates and induce alterations in ROS and oxidative stress.

This *in vitro* study shows contradictory effects of the weak static field exposure depending on the field strength (comprise between 0.5 μT and 600 μT) on various cell functional parameters such as proliferation and ROS production.

Table 1.4.1: Cell studies on exposure to static magnetic fields

Cell type	Endpoint	Exposure conditions	Results	References
Human fibrosarcoma HT-1080 cells n=12 to 63	Oxidative stress, cell growth	0.5 to 600 μT 4 days	Increased or decreased cell growth and oxidative stress on the base of the parameters investigated	Gurhan et al.

2 Extremely low frequency (ELF) fields

2.1 Epidemiological studies

Last years' report concluded that recent epidemiological studies on childhood leukaemia in relation to paternal exposure or residential exposure were in line with previous research: studies indicated an absence of associations with paternal exposure, whereas previously observed associations with residential exposure were observed again in Mexico City. Several studies on adult cancer did not observe increased risks. Regarding Motor Neuron Disease, whether electric shocks rather than magnetic fields (or both) could be relevant, remains unanswered.

2.1.1 Childhood cancer

Seomun et al. [27] undertook a meta-analysis of case-control studies on ELF-MF exposure and risk of childhood leukaemia, childhood brain tumours and any childhood cancer. The study included papers published between 1988 and 2017. In random effect models, when compared to exposure <0.1 μT, exposure ≥0.4 μT was associated with an OR of 1.72 (95%CI: 1.25-2.35) for childhood leukaemia based on 14 studies with a total 37,969 cases of which 101 were exposed to >0.4 μT. Exposure ≥0.4 μT was associated with an OR of 1.25 (95%CI: 0.45-3.45) for childhood brain tumours (6 studies, 21,272 cases, 10 exposed) and an OR of 2.01 (95%CI: 0.89-4.52) for any childhood malignancy (4 studies, 7221 cases, 26 exposed). Risk estimates are ambiguous in the paper, for the present summary numbers were extracted from the figures. The study included only publications included also in previous meta-analyses and confirmed the association with leukaemia but did not provide new insights into what may drive this association.

Amoon et al. [28] investigated ELF-MF exposure and childhood leukaemia by pooling four population-based case-control studies (all also included in the above meta-analysis by Seomun et al.) from California, Denmark, Italy and UK. The study included in total 24,994 cases and 30,769 controls, with the UK study contributing the majority (≈68 %). Except for the small Italian study, exposure was estimated from calculations, 28 cases were exposed to ≥0.4 μT of which 17 were from the Californian study. In unconditional logistic regression, with adjustment for SES, the OR for leukaemia was 0.95 (95%CI: 0.57-1.60) for exposure ≥0.4 μT compared to <0.1 μT. Conditional logistical regression, as used in the original studies, produced similar results (OR 1.07, 95%CI: 0.64-1.78). In country specific analyses ≥0.4 μT was associated with around 50% increased risk in California and Denmark and 50% decreased risk in the UK. In sub-analyses by year, ≥0.4 μT was associated with an OR of 1.54 (95%CI: 0.38-6.28), 1.20 (95%CI: 0.53-2.71) and 0.71 (95% CI 0.32-1.55) in the periods 1953-1983, 1984-1994 and 1995-2010 respectively. In a meta-analysis of these data with the two previous pooled analyses of population-based case control studies, the overall OR for ≥0.4 μT was 1.45 (95%CI: 0.95-2.20). As possible explanations for the observed decline in risk over time within this study and when comparing the results of the three pooled analyses, the authors list changing prevalence of other risk factors for leukaemia, chance and possibly a true risk reduction.

A decreasing risk has previously been reported in the Danish and the UK study whereas it was not observed in the Californian study. The results of this pooled analysis are statistically compatible with the meta-analysis by Seomun et al. It should be noted that, while pooled analysis is a strong approach to utilize existing data, it is no better than the quality of the included studies and may be prone to undue impact of single studies. Whether the observed risk associated with ELF-MF exposure is indeed decreasing remains thus an open question, since it may just reflect a different mix of studies over time.

The international MOBI-kids case-control (Castaño-Vinyals et al 2021), fully summarized in the RF section, found no association with ELF-induced current densities at the centre of gravity of brain tumours in children and young adults aged 10-24.

2.1.2 Adult cancer

Khan et al. conducted a cohort study on ELF-MF exposure from residential transformers and skin cancer (OEM, 2021 [29]) as well as leukaemia and brain tumours (IJHEH, 2021 [30]) in Finland. In their cohort, about 225,000 individuals living in buildings with transformers were included. The average follow-up time was about 15 years. Based on a database of buildings in Finland with indoor transformers, about 4% of people who had lived in an apartment directly above or next to the transformer room for at least six months were grouped as "exposed". Exposed individuals lived either on the ground floor or on the first floor. The remaining residents of the ground floor and first floor and all upper floor residents formed the comparison group. Outcomes were determined by linkage with the Finnish Cancer Registry. Data analysis took into account age at entry, gender and year of birth. Overall, the risk of skin cancer for exposed individuals was not increased, with 559 cases of melanoma and 355 cases of squamous cell carcinoma being evaluated. However, for exposed individuals who lived in the dwellings before they reached 15 years of age, the relative risk for melanoma was increased by a factor of 2.5 (95% confidence interval 1.15 to 5.69) based on seven exposed cases. Haematological neoplasms and brain tumours were not associated with ELF-MF exposure. However, the risk for acute lymphocytic leukaemia was significantly increased (relative risk of 2.9, 95% CI: 1.00–8.2), based on 4 exposed cases. This risk increased with duration of exposure and was particularly associated with childhood exposure (11.5; 95% CI: 1.9–68.9, based on two exposed cases).

Overall, the analysis does not suggest an association between domestic ELF-MF exposure and skin cancer, haematological neoplasms or brain tumours. However, it cannot be excluded that exposure to ELF-MF in childhood could, in very rare cases, favour the development of childhood leukaemia or melanoma. The study approach is innovative as it did not require contact with the study participants and thus selection bias is minimized. In addition, a strength of the study is the exposure assessment, because exposure gradients in buildings with transformers are well established. Only a few confounding factors were considered in the analysis. However, since systematic differences in residential location and transformer room location are unlikely for residents in the same house, this study design implicitly controls for many possible confounders. As such, the study design can be seen as considerably stronger than previous studies addressing residential ELF-MF exposure from high-voltage power lines. For melanoma, the most critical confounder is UV exposure, and it cannot be completely ruled out that children who live on the ground floor and are thus more likely to live close to the transformer room, are also more likely to spend time outdoors, though in a sensitivity analysis limited to study participants living on the ground floor and first floor, no evidence of such a confounding factor was found. The main limitation of the study is the small sample of participants who were exposed during childhood, so confirmation from additional studies is needed.

2.1.3 Neurodegenerative diseases

Filippini et al. [31] performed a systematic review and dose-response meta-analysis on residential exposure to ELF-MF fields from power lines, distance to power lines, and amyotrophic lateral sclerosis (ALS). Six studies were included, of which all had assessed distance to power lines, and two had additionally calculated magnetic field exposure. No increased risks emerged, but the authors acknowledge that risk estimates were very imprecise. Weakness of the study includes the generally low number of exposed cases, which render individual risk estimates very imprecise. In this case here, it also translated into imprecise pooled estimates especially for magnetic field exposures where only two studies could be included. Overall, the study is in line with earlier observations that no increased risks for ALS are observed in residentially exposed persons to ELF-MF from power lines.

Zhao et al. [32] systematically reviewed a range of environmental and occupational exposures (e.g. solvents, air pollutants and greenness) including ELF-MF exposure, and dementia risk. Studies evaluating occupational exposures were meta-analysed and a summary risk estimate of 1.27 (95%CI 1.13-1.43) across 27 studies for exposure to ELF-MF was reported, with high heterogeneity between studies.

As such, the systematic review is in line with previous reports. Due to the brevity of the reporting per environmental exposure, the overall quality of the systematic review is difficult to assess.

Sorahan et al. [33] updated an earlier analysis of a cohort of electric utility workers in the UK (Sorahan 2014) with an additional 8 years of mortality data. Included workers had worked for at least 6 months between 1973 and 1982 as a utility worker. Work histories between 1937 - 1993 were available including information regarding facility/location and job title information. The workplace information was used to calculate individual exposures between 1952 and 1993. Person-years at risk were calculated starting in November 1987 and ended in December 2018. The authors evaluated Motor Neuron Disease as recorded on death certificates and presented risk estimates based on “external” comparisons (standardized mortality ratios were calculated based on the total population of England and Wales, as well as on internal comparisons across exposure groupings). No increased risk in exposed utility workers was observed in external comparisons, although some risk estimates were elevated for more recent exposure in internal comparisons.

As for the previous report, a possible weakness of the study includes the underlying assumption that a person with a specific work title at a specific facility would never change the exact working location (and therefore exposure) for the complete duration of the corresponding job. Such an assumption may have introduced exposure misclassification. Null findings in the comparison with external mortality may be biased due to healthy worker effects and similar patterns were reported in earlier publications on the same occupational group by the author. Previous analyses included 68 cases occurring between 1973-2004, 86 cases when this analysis was expanded to 2010 (Sorahan et al 2007; Sorahan et al 2014). The current analysis included cases starting in 1987 and ended in 2018 and included a total of 69 cases, so fewer than the previous publication. Strength of the analysis include the prospective design, the completeness of the data base and the measurements that were performed to assess ELF-MF exposure at specific workplace locations. It is not well described if workers were still assigned exposures after baseline (the start of follow-up). The total population of England and Wales serves as a comparison group assuming background exposure only. The observation of a possible risk in more recently exposed workers is of interest. The study was funded by the Energy Networks Association, but no statement was provided whether the researchers prevented any undue influence on the study design by the funders.

2.1.4 Other outcomes

Ghazanfarpour et al. [34] performed a systematic review of 17 studies addressing electromagnetic field exposures and (presumably spontaneous) abortion in women. Because exposure sources spanning ELF fields, radiofrequency fields and ionizing radiation were included in the same meta-analytical calculation, and because these fields have strongly different mechanisms of interaction with human bodies, the review is not informative and does not fulfil the quality criteria for a systematic review.

Sahu et al. [35] published a review regarding electromagnetic fields and health. Only free full text publications were included, the search terms included RF-EMF and ELF-MF exposures but were not well specified in terms of exposure sources. Of studies published between 2009 and 2019, only 20 studies were included, of which 8 were original studies and 12 were reviews.

Although the authors described this publication as a systematic review, the complete overview over the current evidence base is hampered by insufficient coverage of search terms, no separation of extremely low frequency vs radiofrequency exposure, experimental vs observational studies, in vivo and in vitro studies. This review is not informative regarding possible effects of electromagnetic fields on human health.

De Souza et al. [36] presented an ecological study performed in Foz do Iguacu, Brazil. All live births between 2012 and 2017 of 327 census sectors were analysed. The authors aimed at evaluating possible socioeconomic risk factors for gastroschisis; as a separate risk factor, presence of a 765 kV power line from the hydroelectric power station in Itaipu was also included. Gastroschisis (ICD10 Q79.3) is a birth defect characterized by abnormal abdominal wall closure with externalization of intra-abdominal structures. The authors applied a range of different spatial (Moran's I, Getis-Ord statistic) and regression analyses approaches (Poisson, logistic regression) to analyse their data. During the observation period, 15 cases of gastroschisis among 26,182 live births were reported. Of the different spatial methods, Getis-Ord identified disease clusters in the study area where also the power lines were located. Logistic regression adjusted for census tract affluence and proportion of young parents (aged <20 years) identified increased risks of gastroschisis if the census tract was close to the power lines (OR 3.5, 95%CI 1.1-10.8).

Limitation of the study is the small number of cases and that the actual exposure of the mothers or parents was not assessed. Potential confounders were accounted for on a census tract level but not at an individual level. Thus, the study may suffer from ecological fallacy, which means that associations on an aggregate level may not reflect associations on an individual level. It is also somewhat unclear what drove the selection of gastroschisis as one sub-category of congenital conditions, and not including also other conditions. There is no clear mechanism linking ELF-MF exposure of mothers to congenital anomalies.

Hansson Mild et al. [37] presented a pilot study of an ELF-MF measurement during a shift in seven train drivers, and in a comparison group of seven men performing “light industrial work”. Average shift exposures (~4 h) were between 2.8 and 8.5 μT. ECG recordings were used to assess heart rate variability, a marker of cardiovascular health. Some small differences in variability were observed in train drivers compared to the industrial workers (lower variability in the low-frequency band and higher variability in the high frequency band). No analyses were performed or presented regarding the actual measured ELF-MF exposures. This study is a pilot study and as such the aim was to test feasibility of the approach, and not the effects of the exposure as such. The presented results therefore do not provide a clear picture as to immediate ELF-MF exposure effects on heart rate variability.

In a cross-sectional analysis, Mohammadi et al. 2021 investigated 110 volunteers from an Iranian iron and aluminium foundry. Exposure to heat and ELF electric and magnetic fields were measured once at the workstation of each participant and testosterone, follicle stimulating hormone and luteinizing hormone were measured from blood samples. In multiple logistic regression, there was no significant correlations between hormone levels and electric or magnetic field levels.

The information provided by the study is limited by the small size, basic analytical approach and the fact that hormones and exposure were only assessed once even if they can exhibit considerable variation over time.

2.1.5 Conclusions epidemiological studies

The current studies do not resolve whether the consistently observed association between ELF magnetic field (ELF-MF) exposure and childhood leukaemia in epidemiology is causal or not.

A new study assessing ELF-MF exposure from in-built transformers indicated possible increased risks of melanoma and ALL in persons who had lived in the higher exposed apartments during childhood. This is an interesting observation that requires follow-up given the small number of exposed cases.

A recent follow-up on an electrical worker cohort on motor neuron disease indicated that possibly more recent exposure to ELF-MF was more relevant than earlier exposures. Electric shocks were not evaluated in this study. The question whether magnetic fields or shocks are underlying the previously observed associations with ALS or motor neuron disease remains unanswered.

2.2 Human studies

In 2021 four studies were published, which investigated ELF effects. Besides the two studies, which were fully discussed in chapter 1.2 (Kursawe et al. 2021, Jankowiak et al. 2021) one study (Okano et al. [38]) investigated effects on hemodynamics, the electrocardiogram, and vascular endothelial function, and another (Evans et al. [39]) the contribution of the location of transcranial electrical current stimulation (tECS) to the perception of phosphenes.

Detection thresholds for alternating current fields (AC) have been investigated by Kursawe et al. (2021). Since the study also included detection thresholds for DC and hybrid electric fields (various DC, constant AC), the study and its pre-study (Jankowiak et al. 2021) have been discussed in detail in the static field section of this opinion (see chapter 1.2 and Table 1.2.1).

In a randomized, double-blind, sham (placebo)-controlled, counterbalanced, crossover trial Okano et al. (2021) investigated the effect of a 15 min 50 Hz MF exposure (at B_{max} 180 mT, B_{rms} 127 mT) on hemodynamics, the electrocardiogram, and vascular endothelial function. They recruited 34 male students as volunteers for two experimental protocols. No information on age of the participants is provided. Protocol A included measurements of blood flow velocity, blood pressure and heart rate, functional near infrared spectroscopy (fNIRS) measurement, and electrocardiogram (ECG) measurement. Data from 10 out of 18 contacted subjects (4 did not reply, 4 were not eligible or did not meet exclusion criteria, respectively) was finally analyzed. Sample size calculation based on pilot data for blood flow velocity revealed that 10 subjects are sufficient to detect a difference of 13.0 cm/s between sham and MF exposure. For protocol B, in which the flow-mediated dilation (FMD), an index of artery endothelium-derived nitric oxide (NO)-mediated vasodilator function was measured, 16 subjects (of which six also participated in protocol A) were included and data analyzed. For both protocols subjects received a sham and an MF exposure twice, the four sessions were scheduled with at least two days between assessments and measurements started after a 10 min rest in a sitting position. For statistical analyses, the mean of the two measurements per exposure condition was used. All trials were performed at constant room temperature (25 °C ± 0.5 °C) and humidity (50% ±10%). The time of testing, however, varied between 11:00 and 17:00 h. Different regional exposures were used for measuring different outcomes: arterial blood pressure, heart rate, fNIRS (15 min; forarm exposure), FMD (30 min; upper arm exposure) ECG (15 min; neck exposure), and blood flow velocity (15 min; forearm, upper arm, and neck). The MF exposure device consisted of two separate electromagnetic coils (distance 160 mm) set horizontally inside the MF exposure device. The value of B_{max} is 180 mT on the surface of the MF exposure device The B_{max} values decrease exponentially with distance. The estimated B_{max} values in an ulnar artery, a brachial artery, and a carotid artery are approximately 13 mT, 8 mT, and 5 mT, in which the distances from the surface of the MF exposure device are approximately 3 cm, 4 cm and 6 cm, respectively. Repeated measures ANOVA with condition (MF exposure vs sham) and time (the number of time points of assessment varies between outcome parameters) were performed. Unfortunately, only the ANOVA results for the interaction term condition*time are reported, which were significant for blood flow velocity (for all three regional exposures), high frequency HF power component of the ECH and FMD and not significant for blood pressure, heart rate, the ration of low-frequency/high frequency power in the ECG, the haemoglobin oxygenation, and the R-R interval. ANOVA results for the factors exposure and time alone are not reported. Pairwise tests for different time points of assessment lead to statistically significant results, p-values, however, were not adjusted for multiple comparisons. Nevertheless, the authors claim that their study provides evidence that a 50 Hz MF can activate parasympathetic activity and thereby lead to an increase in vasodilation and blood flow. Although it is described that measurements were made before and after exposure, it seems that at least some assessments (e.g. blood pressure and heart rate) were also performed while subjects were exposed.

Evans et al. (2021) investigated retinal and cortical contributions to phosphenes using a transcranial electrical current stimulation (tECS). Twenty-two volunteers (11 males and 11 females, aged 19-39 years, mean: 27.4 years) participated in this experimental study. In two sessions scheduled approximately one week apart at similar times of the day, subjects were stimulated with either FPz-CZ (frontal midline – central midline) and Oz-Cz (occipital midline – central midline) montages or a FPz-Oz (frontal midline – occipital midline) and T3-T4 (left and right temporal) montages. The phosphene detection thresholds using an FPz-Oz montage were compared with those from (i) an Oz-Cz montage to determine whether prefrontal regions, such as the retina, contribute to phosphenes and (ii) an FPz-Cz montage to determine whether the visual cortex in the occipital lobe contributes to phosphenes. The order of sessions, the montages within sessions, and the selection of electrodes as cathode or anode were counterbalanced across the subjects. To familiarize the subjects with the appearance of phosphenes, a 10s sinusoidal transcranial direct current stimulation (tDCS) at 1.000 µA first at 16 Hz and then at 22 Hz was used. In the following experiments, seven different frequencies (6, 10, 16, 20, 24, 28, and 32 Hz) were applied in a predetermined random order, which varied across participants and within each montage. Thresholds for phosphene induction were determined for each frequency, using an adaptive staircase procedure. Transcranial AC stimulation started at an intensity of 700 µA, the range was 25 to 1.500 µA, step-size was 25 µA. The choice of anodes and cathodes did not significantly affect phosphene perception thresholds. The 16 Hz frequency produced the lowest mean threshold for all montages. The results indicate that applying tECS over the occipital cortex can affect phosphene perception independent of retinal activation.

Studies published in the current reporting period revealed that low relative humidity (30%) reinforced the perception of AC fields, and that co-exposure of DC and AC electric fields lead to a decrease of the perception threshold compared to either AC of DC alone. Overall, the perception of electric fields was enhanced in the presence of ions. As concluded before (section 1.2.1) this research adds valuable information for a discussion of the acceptability of sensory perception of EF and the so far not existing reference levels and recommendations for hybrid exposure, which is important for political decision-making processes around the construction of high voltage DC power and hybrid power lines. One study found that stimulation of the occipital cortex independently from retinal activation can affect phosphene perception, and another study observed some evidence for an activation of the parasympathetic activity by a 15 min 50 Hz exposure.

Table 2.2.1: Human studies on exposure to ELF fields

Endpoints	Reference	Exposure condition	Sample	Results
Perception threshold of AC and DC EF	Jankowiak et al. (2021)	AC and DC	11 healthy subjects	Please refer to Table static fields human studies
	Kursawe et al. [2021]	AC and DC	203 healthy subjects	Please refer to Table static fields human studies
Hemodynamics, ECG, vascular endothelial function	Okano et al. 2021	50 Hz MF (B_{max} 180 mT, B_{rms} 128 mT)	Exp. 1 10 male students; Exp. 2: 16 male students	Some results indicate that 50 Hz MF can activate parasympathetic activity. The authors claim that this leads to an increase vasodilation and blood flow via a nitric oxide-dependent mechanism
Phosphene perception	Evans et al. (2021)	tECS, 25 – 1500 µA – stepsize 25 µA at 6, 10, 16, 20, 24, 28 and 32 Hz	22 healthy subjects (11 males and 11 females) age range: 19 to 39 years, mean: 27.4 years	The 16 Hz frequency produced the lowest mean thresholds for all montages. tECS of the occipital cortex also contributes to phosphne perception independent of retinal activation.

[1)] SDT: Signal detection theory, [2)] SIAM: Single interval adjustment matrix

2.3 Animal studies

For this reporting year 2021 only one rodent study was identified, whereas seven studies in non-mammalians described effects on honeybees, mosquitos, fruit flies, locusts, and cockroaches.

2.3.1 Physiology and Pathophysiology

Martiñón-Gutiérrez et al. [40] studied metabolic responses of fed or fasted male 10-wk old Wistar rats to a single 15-min ELF-MF exposure. In total, 35 rats (5-12 animals per group) were exposed (60 Hz, 3.8 mT) or sham exposed. Serum levels of glucose, lipids, and indicators of cellular redox state and energy parameters were determined.

A single 15-min ELF-MF exposure caused hyperglycaemia in both fed or overnight fasted rats and led to an attenuated second serum insulin peak. Free fatty acids and lactate serum levels were decreased, but pyruvate and acetoacetate levels increased. In rats previously subjected to 14 days exposure (60 Hz, 3.8 mT, 15 min/d) the above effects were similar.

According to the authors' conclusion, increased serum glucose levels and glucose metabolism induced by a single 15 min exposure were closely related to the cellular redox state and the insulin/glucagon ratio, which was illustrated by correlations among serum glucose levels, insulin, glucagon, and redox-pair metabolites.

2.3.2 Studies in non-mammalians

Agrawal et al. [41] determined effects of acute and chronic exposure to a 75 Hz, 500 µT ELF-MF on three different developmental stages (egg, 3rd stage instar larvae, adult fly) of *Drosophila melanogaster*. For acute exposures 20 eggs, 20 3rd stage instar larvae, and 10m/10f adult flies were exposed continuously for 6 h once. Chronic exposure of 6 h/d for 14 d comprised the complete life cycle of drosophila from egg to adult. Controls were handled accordingly, and experiments were repeated trice.

Acute exposure (1 x 6 h) reduced crawling ability of larvae, and increased ROS levels of larvae and adult flies. Chronic exposure reduced locomotion, eclosion rate, longevity, whereas ROS levels increased. Overall, "maximum effects" were reported after 6 h single exposure in 3rd stage instar larvae and after chronic exposure (egg to adult).

El-Didamony et al. [42] studied in 3rd instar larvae of the mosquito lab strain Culex pipiens various survival limiting parameter following three different 50 Hz ELF-MF (0.25, 0.5, 1 T) exposures for 1 h on 4 successive days. The control and 3 exposure groups consisted of 150 larvae each. Unfortunately, it is not evident whether a real sham control was used. But the larval treatment was similar. Compared to control, total larval body weight decreased significantly in all exposed groups, total lipid content at 0.5 and 1 T. The oxidative stress parameters CAT, GST and MDA were significantly decreased at 1 T, whereas MDA was unchanged at 0.5 and 0.25 T. But CAT and GST activity increased slightly after exposure to 0.5 T, and significantly at 0.25 T. Finally, scanning electron microscopy demonstrated distinct malformations incompatible with survival. The authors conclude that following 0.5 and 1 T exposures the significant alterations in physiological processes and antioxidant system led to severe malformations in all larval body parts. Their outlook towards an electrostatic-based pest control method remains questionable due to practical considerations, especially because of such high field strengths.

Ilijin et al. (2021) published their third paper about the effects of chronic exposure of cockroach nymphs to SMF (see chapter 1.3.3) as well as to ELF-MF (compare Todorovic et al. (2020, 2019), Swedish Radiation Safety Authority [2021, 2022]). Again, one month-old cockroach nymphs (n=20-25 per group) were exposed for 5 months each: (1) 110 mT SMF (see chapter 1.3.3.), (2) 50 Hz, 10 mT ELF-MF, (3) sham exposure. The exposure effects on the insects' relative growth rate (RGR), in brain tissue acetylcholinesterase (AChE) and heat shock protein 70 (HSP70) were investigated. Compared to controls, RGR was significantly increased, whereas AChE activity significantly decreased. By contrast to SMF exposure (see chapter 1.3.3), HSP70 was not affected.

In continuation of their previous work, Migdal et al. [43] looked at behavioural effects of exposure of honeybees to a 50 Hz EF. The same exposure system and EF were used as before

(Migdal et al. [44] , see also Swedish Radiation Safety Authority [2022]). Two-days-old honeybee workers of the following groups: 1) 5.0 kV/m, 2) 11.5 kV/m, 3) 23.0 kV/m, 4) 34.5 kV/m, and 5) control (<2.0 kV/m) were exposed for 1 h, 3 h, 6 h once. Each group consisted of 900 workers per EF and exposure duration, respectively. Five behavioural characteristics (walking, grooming, flight, stillness, contact between individuals, and wing movement) were examined. Overall, the impact of exposure duration on bee's behaviour was demonstrated. Following exposure for 3 h the time that bees spent on behaviours and the number of occurrences decreased. But after 1 h and 6 h, the time and occurrences increased within the groups. The influence of EF on behaviour patterns did not give a clear picture. Concluding, the observations are difficult to interpret. Comparable literature on bee behavioural changes is very rare.

In a second almost identical study Migdal et al. [45] studied bees' behaviour following 12 hours of exposure to 1) 5.0 kV/m, 2) 11.5 kV/m, 3) 23.0 kV/m, 4) 34.5 kV/m, and 5) control (<1.0 kV/m). Each group consisted of 10 cages containing 100 workers each. Acidic, neutral, and alkaline protease levels in haemolymph samples from 100 bees of each group were determined in addition to behaviour. Compared to control, lower number of occurrences of walking, self-grooming, and contacts between individuals were observed in all exposure groups. Moreover, in exposed bees, protease activities were elevated, e.g., at 5.0 kV/m acidic proteases were increased by 78%, neutral by 74%, and alkaline by 40%. Further, no EF strength-dependent increases were seen for acidic and neutral proteases, but not for alkaline. Since increased protease activities were already seen after 1h, 3h, and 6 h of exposure (Migdal et al. [2020b], and when comparing those data with the present study, the time-dependent increase (1, 3, 6, 12 h) still exists for acidic and neutral proteases, but not for alkaline protease.

In summary, the results demonstrate that EF exposure is potentially harmful for bees. Note that worker bees flying at a height of about 2 m near the power line are exposed to an EF of up to 2-10 kV/m, at ca 5 m up to 12 – 15 kV/m. The authors state that they "do not know if the changes in behaviour and protease activity of the honey-bee after E-field exposure persist and for how long".

Migdal et al. [46] reported in their third bee study on biochemical markers in the haemolymph. Again, bee workers were exposed to 50 Hz EF of <1 (control), 5.0, 11.5, 23.0, or 34.5 kV/m for 1, 3, 6, 12 h. Haemolymph samples (n=100/gr) were taken immediately after exposure stop. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) decreased in all EF groups and was the lower the longer the exposure time. The researchers conclude that the 50 Hz EF may impair crucial metabolic cycles (e.g., citric acid cycle, ATP synthesis, oxidative phosphorylation, β-oxidation). Also, the concentration of albumin and creatinine was affected, but not consistently in one direction. E.g., after 5.0 and 11.5 kV/m EF, albumin was higher the longer the exposure time, and creatinine lower the longer the exposure time. No significant changes of albumin and creatinine concentrations were seen following one hour-exposure to 11.5, 23.0, 34.5 kV/m. The albumin and creatinine changes "may indicate a disturbance in protein metabolism and increased muscle activity".

According to all five studies recently published by Migdal et al. (2020 a, 2020 b, 2021 a-c), biochemical parameters in haemolymph, the antioxidant system and behaviour of honeybees are affected when applying 50 Hz EF as low as 5.0 or 11.5 kV/m (which is environmentally relevant) and higher EF.

Finally, Shephard et al. [47] tested the influence of ELF-MF on locust wingbeats. Using high-speed video recording the wingbeat frequencies of individual locusts tethered between a pair of copper wire coils generating EMFs of various frequency were analysed.

- Groups of 20 locusts were exposed for 15 s to a 50 Hz ELF-MF of 0 (=sham control), 100, 1000, and 7000 µT (The range of ELF-MF reflected those found in the environment close to high voltage transmission lines (Shephard et al., 2021)). 2) The effects of EMF frequency were tested in a field where the MF strength was kept constant at 700 µT and 17, 20, and 22 Hz (n = 21, 20, and 19 respectively) were applied for 15 s. The mean (pre-exposure) wingbeat frequency of tethered locusts was 18.92 ± 0.27 Hz.
- Acute 15 s exposure to 50 Hz EMFs significantly increased absolute change in wingbeat frequency in a MF strength-dependent manner, i.e., greater MF led to greater changes in wingbeat frequency. Moreover, the initial (pre-exposure) wingbeat frequency of a locust is important for the direction of change. Locusts flying at <20 Hz increased their wingbeat frequency, while those with >20 Hz decreased it.
- Compared to pre-exposure, during exposure of 17, 20, and 22 Hz the wingbeat frequencies changed close to but did not reach normal wingbeat frequency of 18.9 Hz. Overall, the study demonstrated that exposure to ELF-MF caused small but statistically significant changes in wingbeat frequency of locusts, and that locusts entrained to the exact frequency of the applied ELF-MF.

2.3.3 Summary and conclusions on ELF animal studies

In contrast to the previous Council reports, only one rodent study was identified. Hyperglycaemia in fed or fasted rats was reported following a single 15 min lasting ELF-MF exposure at 60 Hz and 3.8 mT.

Further three studies in honeybees addressing ultra-high voltage (UHV) transmission technology and using ELF-EF (50 Hz, 5 – 34.5 kV/m) resulted in a decrease of behavioural characteristics such as walking, grooming, flight, stillness, contact between individuals, and wing movement. In haemolymph of bees, protease activities were increased, whereas alkaline phosphatase and the aminotransferases ALT and AST decreased exposure time-dependently. Summarizing this and the previous Council report, haemolymph, antioxidant system and behaviour of honeybees are affected when applying 50 Hz EF as low as 5.0 or 11.5 kV/m.

A further study in cockroach nymphs exposed to 50 Hz, 10 mT ELF-MF showed after 5 months an increased body weight development and a decrease in brain acetylcholine esterase.

In fruit flies exposed to 75 Hz 500 µT ELF-MF reactive oxygen species were increased, whereas locomotion, eclosion rate and longevity decreased. Following 50 Hz exposures up to 1 T in mosquito larvae severe malformations were detected.

Finally, 20 Hz and 50 Hz ELF-MF caused small but significant changes in wingbeat frequency of locusts, and locusts were entrained to the exact frequency of the applied ELF-MF.

In this reporting year, various effects were again reported after ELF-MF exposure in different animal models, mostly non-mammalian. For the most part, plausible mechanisms are still unclear.

Table 2.3.1: Animal studies on exposure to ELF fields

Endpoint	Reference	Exposure conditions	Species & Strain	Results
Rodent studies				
Physiology & Pathophysiology	Martiñón-Gutiérrez et al. (2021)	60 Hz, 3.8 mT 15 min once 15 min, 14 d	Wistar rat	Hyperglycemia related to the cellular redox state and the insulin/glucagon ratio
Studies in Non-mammalians				
Development, locomotion, oxidative stress	Agrawal et al. (2021)	75 Hz 500 µT 6 h once 6 h, 14 d	Drosophila melanogaster	ROS increased. Locomotion, eclosion rate, longevity decreased.
	El-Didamony and Osman (2021)	50 Hz, 0.25, 0.5, and 1 T 1 h, 4 d	Mosquito larvae, (*Culex pipiens*)	BW, total protein, lipid content decreased. Oxidative stress parameters exposure-dependently affected. Malformations at 0.5 and 1 T.
(Patho)Physiology, Behaviour and locomotion	Ilijin et al. (2021)	50 Hz 10 mT 5 mo	Cockroach (*Blaptica dubia)*	RGR increased brain AChE decreased
	Migdal et al. [48] (2021a)	50 Hz 5, 11.5, 23, and 34.5 kV/m 1, 3, and 6 h	Honeybee *(Apis mellifera carnica)*	3 h: time that bees spent on behaviours decreased but increased after 1h and 6h. No clear EF dependency.
	Migdal et al. (2021b)	50 Hz 5, 11.5, 23, and 34.5 kV/m 12 h	Honeybee *(Apis mellifera carnica)*	Decreased behaviours (occurrences of walking, self-grooming, contacts between individuals). Protease levels in haemolymph increased.
	Migdal et al. (2021c)	50 Hz 5, 11.5, 23, and 34.5 kV/m 1, 3, 6, and 12 h	Honeybee *(Apis mellifera carnica)*	Time-dependent decrease of AST, ALT, and ALP in haemolymph.
	Shephard et al. (2021)	50 Hz: 0.1, 1, 7 mT; 15 s 17, 20, 22 Hz: 700 µT; 15 s	Locust *(Schistocerca gregaria)*	Locusts entrained to the exact frequency of the applied ELF-MF.

Abbreviations: AChE: acetylcholinesterase; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; bw: body weight; d: day(s); h: hour(s); mo: month(s); RGR: relative growth rate; ROS: reactive oxygen species.

2.4 Cell studies (ELF)

Cell studies were carried out on several cell types of different origins (human, canine and murine), dealing with proliferation, DNA damages, ROS production and circadian rhythm. Compared to the previous Council report a similar number of studies was identified for the reporting year 2021.

2.4.1 Proliferation

The effect of 1 and 24 h exposure to 50 Hz (0.2 and 0.4 mT) and of co-exposure with Di (2-ethylhexyl) phthalate (DEHP), known as environmental endocrine disruptor, on the proliferation of human amniotic cells (FL) was investigated by Chen et al. [49]. Exposures were done in blind-manner and temperatures were controlled throughout the protocols. The results of four independent experiments showed that 0.4 mT MF exposure alone for both 1 h and 24 h promote the proliferation of FL cells, whereas 0.2-mT MF exposure has no influence if compared to sham-exposed cells. At concentration of DEHP (0.1 μM) that could not promote FL cell proliferation, co-exposure with 50-Hz, 0.2-mT MF for 1 h or 24 h increase significantly the proliferation rate of cells compared to single factor exposure (DEHP or ELF alone). The activation of proteins known to be involved in activating proliferation-related signal pathways such as kinase B (Akt), sphingosine kinase 1 (SphK1) and extracellular signal regulated kinase (ERK) seemed to be link with the synergetic effect of DEHP and ELF.

In the paper of Domínguez et al. [50], transepithelial electrical impedance (TEEI), which includes transepithelial electrical resistance (TEER) and cellular capacitance (Ccl), and gives information about the barrier properties of cells grown on permeable membranes was assessed in the Madin–Darby Canine kidney (MDCK) cell line (model of epithelium cells) exposed to 60 Hz (1 and 5 mT) 60 min every 4 h, over 24 h. Temperature was monitored during the experiments. Cell migration and proliferation using the scratch-wound assay, expression of the tight junction proteins Claudin-1 (CLDN1) and Zonula occludens-1 (ZO-1), and changes in the size and shape of the cell nuclei were also followed. No significant effects on TEEI values and in migration and proliferation were observed when 1 and 5 mT 60 Hz magnetic fields were applied to confluent cell monolayers (12 independent experiments). The expression of CLDN-1 decreased at 5 mT by 90% compared with the control. The expression of ZO-1 increased by 120% during the first 24 h of magnetic field exposure at 5 mT (in a tested sequence of 24 h on, 24 h off cycle). The results of six independent experiments showed no significant effects in the size and shape of the cell nuclei, but significant differences were seen in their density. The authors concluded that exposure to a 60 Hz magnetic fields might have effects on MDCK epithelial cells.

Heidari et al. [51] have investigated in human adenocarcinoma gastric cancer cell line (AGS), the changes in the expression of B-cell lymphoma 2 (BCL2), of microRNAs miR-15-b and miR-16 following the exposure to magnetic flux densities (MFD) of 0.2 and 2 mT given continuously and with 1.5 h on/1.5 h off cycles for 18 h. BCL2 is an anti-apoptotic protein and miR-15-b and miR-16 are key regulators of BCL2. MiRNAs can act as oncogenes or tumor suppressors in a variety of cancers, including gastric cancer. Changes in the cell viability were evaluated by the MTT assay. Real- time PCR was used to evaluate the expression changes of BCL2, miR-15-b and miR-16. The results of three independent experiments showed that ELF-EMF could significantly reduce the viability of AGS cells in the continuous MFD of 2 mT. The BCL2 expression was significantly decreased following the exposure to continuous MFDs of 0.2 and 2 mT and discontinuous MFD of 2 mT. The expressions of miR-15-b and miR-16 were significantly increased in continuous and discontinuous MFD of 2 mT. According to the results, ELF can change the expressions of BCL2, miR-15-b and miR-16. Given the role of BCL2 in apoptosis and the role of miR-15- b and miR-16 in its regulation, the overexpression of regulatory miRNAs and a concomitant reduction of BCL2 expression under the exposure of ELF- EMF could be effective in driving cancer cells toward apoptosis and in controlling the growth of AGS cells.

2.4.2 Other cellular endpoints (DNA damage, ROS production, Circadian rhythm)

Cells from three different systems or organs, including the reproductive system (Human amnion epithelial cells FL), endothelial system (human umbilical vein endothelial cells HUVECs), and skin (human skin fibroblast cells (HSFs) were used in this study (Lv et al. [52]). DNA damage after exposure to 50 Hz at 0.4, 1, and 2 mT for 15 min, 1 h, and 24 h, were evaluated using the earlier γH2AX marker to visualize DNA double-strand breaks (DSBs). The results of three independent experiments for each cell line showed that there was no significant change in γH2AX by either immunofluorescence or western blot in all cell types and whatever the exposure conditions (if compared to sham-exposed cells).

In previous studies, Martinez et al. reported that proliferation of the neuroblastoma cell line NB69 was increased by ELF exposure through activation of signal pathways MAPK-ERK1/2 and p38 (Martinez et al, 2012, 2016 and 2019). In the present study (Martínez et al. [53]), the possible involvement of the NADPH oxidase, main source of ROS in this activation is hypothesized. Tests were conducted with and without Diphenyleneiodonium (DPI), used to inhibit NADPH oxidase and N-acetyl-L-cysteine, used as ROS scavenger. The expression of the NADPH oxidase component p67phox subunit was also assessed. Exposure to the magnetic field 50 Hz for 10 min, 100 µT significantly increase the level of ROS and the expression of p67phox compared to the sham exposure (three independent experiments). The activation of MAPK-JNK pathway after 20 min magnetic field (MF) exposure was prevented by DPI but not that of ERK1/2 or p38 pathways. The authors concluded that 50 Hz MF might increase ROS production via NADPH oxidase and different mechanisms were involved in the 50 Hz magnetic field proliferative response.

Mustafa et al. [54] investigated the effects of 50 Hz on gene expression related to the circadian rhythm or DNA damage signalling and whether these fields modify DNA damage repair rate after bleomycin treatment. The circadian rhythm plays a vital role in regulation of major cellular activities and anomalies in these activities can be linked to cancer development and progression. Murine Factor-Dependent Continuous - Paterson 1 cells (FDC-P1) hematopoietic cells were exposed at 200 µT MF for different durations (15 min, 2 h, 12 h, and 24 h) or sham-exposed (three independent experiments). To assess DNA-damage signalling and DNA repair rate, the cells were subsequently treated with 20 µg/mL bleomycin for 1 h and then either assayed immediately or allowed to repair their DNA for 1 or 2 h. Circadian rhythm-related genes were upregulated after 12 h of MF exposure and downregulated after 24 h of MF exposure, but none of the affected genes were core genes controlling the circadian rhythm. The DNA-repair rate for bleomycin-induced damage was only decreased after MF exposure for 24 h. These results suggest that the effects of 50 Hz MF are exposure duration-dependent.

Table 2.4.1: Cell studies on exposure to Extremely low frequency (ELF) fields

Cell type	Endpoint	Exposure conditions	Results	References
Human amniotic (FL) cells n= 4	Proliferation	50-Hz 0.2 or 0.4 mT 1 h or 24 h Co-exposure with DHEP	0.4 mT for both 1 h and 24 h promote the proliferation. No influence of 0.2-mT. DEHP (0.1 μM) + 0.2-mT 1 h or 24 h increase significantly the proliferation.	Chen et al., (2021)
Madin–Darby Canine kidney (MDCK) n=12 TEEI n=6 wounds and immunofluorescence	Proliferation-Migration	60 Hz, 1 mT and 5 mT 60 min every 4 h, over 24 h (six exposures) For tight junction proteins ZO-1 and CLDN-1 24 h exposure, 24 h no exposure, 24 h exposure	No effects on TEEI, migration and proliferation. No effects in the size and shape of the cell nuclei, but significant differences in their density. Effects on tight junction proteins.	Domínguez et al., (2021)
Human adenocarcinoma gastric cancer cell line (AGS) n= 3 (duplicate)	Proliferation-Apoptosis	50 Hz, 0.2 and 2 mT continuously and discontinuously (1.5 h on/1.5 h off) for 18 h, Solenoid	Decrease viability in the continuous MFD of 2 mT. Decreasedof BCl2 expression following the exposure to continuous MFDs of 0.2 and 2 mT and discontinuous MFD of 2 mT. Increasedexpressions of miR-15-b and miR-16 in continuous and discontinuous MFD of 2 mT.	Heidari et al., (2021)
Human amnion epithelial cells (FLs), human skin fibroblast cells (HSFs), and human umbilical vein endothelial cells (HUVECs) n= 3	DNA damage	50 Hz, 0.4, 1, and 2 mT for 15 min, 1 h, and 24 h, Helmholtz coils	No effect.	Lv et al., (2021)
Human neuroblastoma NB69 cell line n= 3 (4 replicates)	ROS production	50 Hz, 100 µT 5-30 min,	Increase in the level of ROS and the expression of p67phox.	Martínez et al., (2021)
Murine Factor-dependent continuous -Paterson 1 cells (FDC-P1) n= 3	Circadian rhythm	50 Hz, 200 µT 5-30 min, 5 min, 2, 12 and 24 h	Upregulation of circadian rhythm- related genes after 12 h of exposure and downregulated after 24 h. DNA-repair rate for bleomycin-induced damage decreased after MF exposure for 24 h.	Mustafa et al., (2021)

CLDN-1: Claudin 1; DHEP: Di (2-ethylhexyl) phthalate; TEEI: transepithelial electrical impedance; ZO-1 : zonula occludens -1

2.4.3 Conclusions on ELF *in vitro* studies

Six *in vitro* studies on ELF-EMF exposure have been included in the report, which does not allow any firm conclusions regarding possible biological effects from exposure to ELF-EMF. Two more studies had to be excluded due to insufficient number of experiments or description of the exposure system.

3 Intermediate frequency (IF) fields

3.1 Epidemiological studies

The previous report stated that only one new study on IF-MF had been published, precluding conclusions regarding possible health effects from exposure to IF-MF. The same applied to this year's report.

Tokinobu et al. [55] performed a survey among 1565 women living on Kyushu Island, Japan. Participating women filled in questionnaires between the 5th and 39th week of pregnancy, as well as after delivery. Women were asked if they used induction cooking at home as their main kitchen appliance, which was the case for 14% of the women. The authors evaluated associations with birth outcomes (preterm birth, low birth weight, small for gestational age), after adjustment for a range of potential confounders. Women using induction cookers had a reduced risk of preterm birth, the other birth outcomes were not associated with induction cooking.

Only few previous studies have been performed that assess intermediate field exposures and any type of health effects in humans. Strength of the study include the design including women during pregnancy. Weaknesses include that no exposures were measured, that a response rate could not be determined, and that some degree of selection bias may have affected results. In particular, authors reported that women using induction cooking were on average a bit older, a bit more likely to already have children, and to be somewhat (borderline) less likely to be employed. Although risk estimates were adjusted, it is conceivable that residual confounding, or chance, may have introduced the observed seemingly "protective" effect.

A single study on IF-MF exposure and pregnancy outcomes was published in the relevant reporting period; the study does not allow firm conclusions regarding possible health effects from exposure to IF-MF.

3.2 Human studies

As for the previous reporting periods, there was no human experimental study in the intermediate frequency range.

3.3 Animal Studies

Two animal studies were identified for the current reporting year 2021.

3.3.1 Behaviour

Lerchl et al. [56] continuously exposed 3-mo. old female CD1 mice (n=80 / group) to 20 kHz 360 µT (rms) or sham for 10 months in Helmholtz coil systems. Endpoints were body weight development, survival rate, and occurrence of tumours in brain, liver, kidney, spleen, lung, and lymphatic tissue at 13 months of age. After 7 months of exposure, animals' behaviour was evaluated using three different test systems. Compared to sham, body weight development and survival was similar. In the behavioural tests, the 8-arm maze test did not show differences. But Rotarod data demonstrated significantly longer retention times of IF exposed mice, and open field trials revealed a significantly lower number of supported rears (i.e., rears with contact to the wall of the open field arena). The other open field endpoints (presence in the centre and the peripheral squares, unsupported rearing, cleaning, and defecation) did not differ. Finally, the observed tumour incidences were not significantly different between sham and exposed mice.

The authors discuss the behaviour effects in terms of missed food deprivation before testing, animals' age when tested, and mouse strain. Overall, they conclude "that there is a lack of evidence that IF-exposure contributes to any significant behavioural changes". But in the paper's abstract they state, "the significant differences in the behavioural tests may indicate higher levels of alertness in mice". This is contradictory. Unfortunately, the same holds true for their summarizing "no adverse effects of exposure …on tumour incidences". Compared to other cancer studies they correctly discuss limited histopathology and too short study duration. Nevertheless, they address the lack of carcinogenicity what cannot be deduced from this study but from others including Nishimura et al. [57] (see Swedish Radiation Safety Authority [24]).

3.3.2 Genotoxicity

Ohtani [58] et al. analysed in blood of male C57BL/6J mice genotoxicity, biochemical and haematological parameters following a 2-wk, 5 d/wk, 1 h/d lasting IF-MF exposure. The mice received an average whole-body EF of 54.1 V/m (which is -2.36 x the basic restriction for occupational exposure according to ICNIRP) by a system generating 25.3 mT flux density at 82 kHz (which is is relevant for wireless power transfer systems for charging electrical vehicles). Micronuclei (MN) were determined in reticulocytes and mature red blood cells before (pre), on days 0, 2,3,6,10, and 14 after the 2-wk IF-MF or sham exposure (n=5 mice/gr). Accordingly, the *Pig-α* mutation assay was performed pre, and on days 2, 7, and 14. (Note: The *Pig-α* mutation assay detects the frequencies of reticulocytes or mature erythrocytes harbouring a mutated *Pig-α* gene. If the Pig-a locus on the X chromosome is defective red blood cells become haemolytic. The *Pig-α* mutation or PIGRET assay effectively detects *in vivo* mutagenicity following exposure to radiation, cyclophosphamide etc.) Blood chemistry and blood cell analyses were performed in n=8 mice per group.

Body weight development, 15 parameters of blood cell analysis, and 14 parameters of blood chemistry were similar between exposed and sham mice. MN tests and *Pig-α* mutation assay did not reveal any significant differences between IF-MF- and sham-exposed. Concluding, the above tests do not show genotoxicity or general toxicity (as demonstrated by blood analysis) following an IF-MF exposure of 82 kHz and 15.3 mT. In addition, the authors point out that the above genotoxicity data support findings from their previous study in which analysis of microarray data from both the brain and liver did not show significant differences in transcriptional expression (Ohtani et al. [59], Swedish Radiation Safety Authority [2021]).

3.3.3 Summary and conclusions on IF animal studies

In the 20 – 100 kHz range, in total two mouse studies did not result in adverse effects on behaviour and genotoxicity.

Table 3.3.1: Animal studies on exposure to IF fields

Endpoint	Reference	Exposure conditions	Species, strain, sex	Results
Behaviour	Lerchl et al. (2021)	20 kHz 360 µT 24 h/d, 7 d/wk, 10 mo	Mouse, CD-1, female	No significant behavioural changes after 7 months. No differences in tumour incidences of selected organs following 10 month- exposure. (Note: Too short study duration!)
Genotoxicity	Ohtani et al. 2021)	82 kHz, 25.3 mT 1 h/d, 5 d/wk, 2 wk	Mouse, C57BL/6J, male	No genotoxicity and general toxicity determined in blood (cells)

Abbreviations: d: days; h: hour(s); IF: intermediate frequency, MF: magnetic field; MN: micronucleus; mo: month(s); wk: week(s).

3.4 Cell Studies

Only one paper has been included in the report, dealing with the exposure of porcine oocytes to evaluate maturation and embryonic development.

Chen et al. [60] used porcine oocytes as a model system to evaluate the effect of 40 kHz at intensities of 0 (sham), 2.5, 5, 7.5, or 10 mT on the *in vitro* maturation and the subsequent embryonic development. Due to the design of the EMF device, the samples were exposed at different intensities simultaneously and a water jacket, filled with circulating water, stabilized the culture temperature at 39°C. The results of at least three experiments showed no significant differences in maturation rates. To confirm the cytoplasmic maturation of oocytes, mitochondrial distribution, cytoskeleton distribution, autophagy and early embryonic development were evaluated. No differences in cytoskeletal distribution patterns were detected among sham and exposed samples, while oocytes in the 2.5 mT group had a significantly lower proportion of mature mitochondrial patterns than the sham-exposed and the other exposed groups.

Autophagy was evaluated by measuring the expression of two proteins: the microtubule-associated protein 1A/1B-light chain 3 (LC3), a central protein in the autophagy pathway, and the mechanistic target of rapamycin mTOR. No significant differences in mTOR were detected in all cases, while both the 2.5 mT and the 5 mT-treated groups had increased mean fluorescence intensities of LC3B compared to sham controls. Early embryonic development was measured by evaluating cleavage and blastocyst rates.

Compared to sham-exposed samples, a statistically significant decrease for both parameters was detected in 2.5 mT- and 5 mT-treated groups but not in 7.5 mT and the 10 mT-treated groups, but the total cell numbers of day 7 blastocysts was unaffected. From the results here reported it appears that exposure to lower intensity IF-EMF could interfere with oocyte maturation. In this study the authors refer to the exposure duration in terms of in vitro maturation, but the exposure time is not reported.

Table 3.4.1: Cell studies on exposure to IF fields

Cell type	Endpoint	Exposure conditions	Results	References
Porcine oocytes n= at least 3 independent experiments	mitochondrial distribution, cytoskeleton distribution, autophagy and early embryonic development	40 kHz 2.5, 5, 7.5, or 10 mT	Lower proportion of mature mitochondrial patterns in 2.5 mT exposed samples; increased LC3B expression in 2.5 and 5 mT exposed samples; decreased early embryonic development at 2.5 and 5 mT. No effects in maturation rate, cytoskeletal distribution patterns and cell number of blastocysts.	Chen et al. (2021)

Abbreviations: LC3: microtubule-associated protein 1A/1B-light chain 3.

Only one new study on IF-MF has been included in the report, which does not allow any firm conclusions regarding possible health effects from exposure to IF-MF. Six more studies have been found but they had to be excluded due to methodological limitations.

4 Radiofrequency (RF) fields

4.1 Epidemiological studies

Last years' report concluded that little new research had been published on tumour risk in relation to mobile phone use. A French study found an association between maternal mobile phone used and foetal growth restriction but retrospectively collected mobile phone use data was a limitation for this study. A new analysis from the COSMOS cohorts including more than 24,000 subjects found weak indications for an association between mobile phone use and sleep quality and suggested that other factors than RF-EMF may explain the observed association. A French cross-sectional study did not observe associations between RF-EMF from base station and non-specific or insomnia-like symptoms. New studies in adolescents on cognitive functions and brain volume did not indicate increased risks from RF-EMF exposure.

4.1.1 Childhood cancer

The international MOBI-kids case-control study with participation from Australia, Austria, Canada, France, Germany, Greece, India, Israel, Italy, Japan, Korea, the Netherlands, New Zealand and Spain, investigated use of wireless phones and risk of brain tumours among children and young adults aged 10-24 (Castaño-Vinyals et al. [61]). They recruited 899 cases, hospitalized with a tumour located in the brain, excluding the most central parts, which were deemed situated too far from the source of exposure to experience appreciable exposure. The exact location of the tumour in 3D within the brain was determined from MRI. Controls (n=1910), matched on sex, age, geographical region and date of interview, were recruited among patients admitted to hospital for appendicitis. The participation rates for cases and controls were 72% and 54% respectively. Cases and controls answered an administered questionnaire including detailed questions on use of cordless and mobile phones. Exposure was assessed as years since first use, cumulative number of calls and cumulative call time. Additionally cumulative RF energy and current density at tumour location were estimated using an elaborate algorithm. Neuro-epithelial tumours (n=671), primarily glioma and non-neuro-epithelial tumours (n=129) were analyses separately using conditional logistic regression, with adjustment for parental education. There was no evidence of increasing risk with increasing level of exposure and in many cases, there were suggestions of an inverse association. E.g. 10 or more years of mobile phone use was associated with an OR of 0.75 (95%CI: 0.5-1.13) for neuro-epithelian tumours when compared to those using a phone for less than a year. The results were robust in a range of sensitivity analyses. However, excluding proxy-interviews or excluding use in the 5-year period prior to diagnosis/index to account for potential prodromal symptoms brought risk estimates closer to the null. The authors therefore suggest that the decreased risks likely reflect bias in the proxy interviews and possibly prodromal syndromes of brain tumours and they conclude that there was no evidence of an association between wireless phone use and brain tumours among young people. The authors note that since there are few established risk factors for these tumours they cannot rule out that residual confounding may have influenced their results. Limitations include the small number of cases for subgroup analyses. Exposure assessment was based on self-reports. Assessment of RF and ELF-MF exposure at site of tumour origin is novel and innovative but based on many assumptions leading to imprecision which may have driven risk estimates towards the null. By design, case-controls studies are prone to a range of biases e.g. relating to participation, recall and reporting. The subset analyses in this study minimizing these issues are in line with incidence trend studies that do not indicate increases in brain tumours corresponding to the huge increase in exposure from mobile phones.

4.1.2 Adult cancer

Rodrigues et al. [62] performed an ecological study in Brazil on radiation from mobile phone base stations and cancer mortality. For each one of 27 Brazilian province capitals they estimated annual exposure to radiofrequency radiation from the number of operating base stations in the years 2010-2017 and for the same period calculated age and gender specific cancer-mortality rates. In Poisson regression models adjusting for age, calendar year, capital and capital specific area and GDP, log(base station years/ km^2) was statistically significantly associated with mortality from all cancer (RR: 1.15), breast cancer (RR: 1.25), cervix cancer (RR: 2.18), lung cancer(RR: 1.14), oesophagus cancer (RR: 1.18) and brain cancer (RR: 0.86). Confidence intervals were not reported. The authors conclude that radiation from base stations increase the rate of death for all types of cancer.

The foremost limitation of this study is the extremely crude exposure assessment and the ecological study design, which is vulnerable to ecological fallacy. Other limitations include the reliance on mortality data and very limited adjustment for confounders. The proportion of people in each city with any appreciable exposure from the base stations will be negligible and given that more detailed studies on exposure from base stations do not indicate associations with the analysed outcomes, it seems unlikely that any effect on cancer mortality could be large enough to be detectable on population level. Extrapolating these findings to effects from mobile phones, which cause much higher exposure on a population level, would mean that these types of cancers would have substantially increased over time, which is not the case. The study results must thus be considered as implausible.

Unilateral hearing loss is a primary symptom of vestibular schwannoma (AKA acoustic neuroma). Hephziba et al. [63] therefore hypothesized that because use of a mobile phone is a mono-aural task it could possibly lead to earlier detection of this disease. They performed a retrospective questionnaire study, of 61 patients admitted to a neurosurgical clinic with a vestibular schwannoma, between 2017 and 2022. Patients with a personal mobile phone (n=44) used it more and had a shorter duration of hearing loss prior to diagnosis, than those with a shared phone (n=16). Only one participant had no phone at all. They compared their results with a similar, previous study of 50 patients, diagnosed between 2003 and 2005 [64]. Patients in the earlier study were less likely to use a mobile phone (48% vs 97%), had larger tumours (3.9 cm vs 3.3 cm) and they tended to have longer duration of hearing loss preceding diagnosis (47 months vs 29 months). The authors concluded: “Increased call phone use has led to earlier diagnosis of VS”.

Although mobile phones may be used in more noisy circumstances, use of landline phones is equally mono-aural and these are not addressed in the study, it is thus unclear to what extent the basic assumption that the increased use of mobile phones might lead to earlier detection in the population holds true. In addition, the study does not explore alternative explanations for the observed differences. As an example, the quality of health care and the diagnostic capabilities available are likely to differ between the two periods and possibly between people with their own phone and people with a shared phone. Further, the sample size is small.

Karipidis et al. [65] examined incidence time trends of parotid and other salivary gland cancers in Australia to evaluate whether incidence increased after introduction of mobile phones. Annual percentage incidence changes were estimated using Poisson regression based on all available national registration data from 1982 to 2016, as well as for specific time periods (1982-1993, 1994-2005, 2006-2016) representing changes in mobile phone use of the Australian population (little use, strong increase, majority use). Over the whole period, the incidence of parotid gland cancer was decreasing for men and increasing for women aged 20 to 59 years. Strongest trends were observed for 2006 - 2016 with a 3.7% (95 %CI: -6.7% to -0.7%) annual decrease in parotid gland cancer for males and a 4.8% (95% CI: 1.8–7.9%) increase in females aged 20-59 years. The incidence for other salivary gland cancers was stable during all the periods. The authors conclude that the results do not indicate an increased risk for parotid or other salivary tumours from mobile phone use. The observed increase in parotid gland cancer in females may be attributed to other possible risk factors specific to this gender, though risk factors for parotid gland cancer are largely unknown except high doses of ionizing radiation and possibly smoking.

Incidence time trend studies are useful to detect risk increase for diseases with external risk factors varying little over time. Of the salivary glands, the parotid glands are closest to a mobile phone while talking and thus relatively highly exposed.

Temporal changes in mobile phone subscriptions and the incidence of glioma in Canada between 1992 and 2015 were evaluated by Villeneuve et al. [66], based on data from the Canadian Cancer Registry. Time trends were compared with risk predictions from previous case control studies: i) a recent pooled analysis of Swedish case-control studies, ii) the 13 country INTERPHONE study, and iii) more recent results from data collected from the Canadian component of the INTERPHONE. Age-standardized glioma incidence rates were found to be stable between 1992 and 2015 and predictions using the three case-control studies were found to overestimate the observed number of glioma cases diagnosed in Canada in 2015 by 50%, 86%, and 63%, respectively. The authors concluded that observed time trends of glioma incidence are not compatible with an increased risk from mobile phone use as reported in the three case-control studies.

Although small risks cannot be excluded with such time trend studies, the result indicate that a substantially increased brain tumour risk as reported in some case-control studies are implausible and most likely the result of bias, such as differential recall of previous mobile phone use by brain tumour patients and healthy individuals. If the risks observed in the discussed case-control studies reflected true risks, a substantial increase in the incidence of brain tumour should have occurred. This has not been observed in cancer registries, and given the few established external risk factors for brain tumours, it is unlikely that an increase in risk from mobile phone use has been masked by other secular time trends.

Withrow et al. [67] evaluated the incidence of non-malignant meningioma and vestibular schwannoma from 2004 through 2017 in the United States using data from the Surveillance, Epidemiology, and End Results (SEER). Annual percentage changes (APC) were estimated using log-linear models. Based on 108,043 cases of meningioma an APC increase of 5.4% (95% CI: 4.4%-6.4%) was observed from 2004 to 2009 with a subsequent slower increase afterwards (1.0%; 95% CI: 0.6%-1.5%). Microscopically confirmed (MC) meningiomas changed little from 2004 to 2017 but radiographically confirmed (RCG) meningiomas rose by 9.5% (95% CI: 7.8%-11.1%) until 2009 and thereafter by 2.3% (95% CI: 1.5%-3.0%). Overall vestibular schwannoma rates (n = 17,475) were stable (APC, 0.4%; 95% CI: −0.2%, 1.0%), but MC vestibular schwannoma rates decreased (APC, −1.9%; 95% CI: −2.7%, −1.1%), whereas RGC vestibular schwannoma rates rose (2006-2017: APC, 1.7%; 95% CI: 0.5%-3.0%). Generally, all trends were similar for each sex and each racial/ethnic group, but RGC diagnosis was more likely in older patients and for smaller tumours. The authors concluded that the recent stable rates argue against an association with mobile phone use, although overall trends were obscured by differences in diagnostic methods.

With the same limitations as discussed above, this study is in line with other time trends analyses, which do not indicate that tumours of the head increased after widespread use of mobile phones. The study suggests that diagnostic praxis may introduce spurious time trends, which needs to be taken into account when interpreting such ecological analyses.

Choi et al. [68] evaluated brain cancer incidence trends in Korea, in the period 1999-2017. Age and sex standardized incidence rates (ASR) were calculated using data form the Korean Statistical Service and the Korean national cancer registry. In total 29,721 patients with cancer of the brain or CNS, excluding spinal cord and unspecified locations were observed. The ASR of glioma and glioblastoma increased over the study period, particularly among the elderly, this was matched by a decreasing incidence of unspecified brain tumours and the overall ASR (2.89/100.000) of brain cancer was constant throughout the study period. Similarly, the proportion of tumours of unspecified location decreased whereas tumours located in the frontal and temporal lobe increased. The authors also calculated predicted incidence rates under different scenarios of brain tumour risk associated with mobile phone use. The annual proportion of Koreans using a mobile phone since 1989 was estimated from national data on number of mobile phone subscriptions in Korea. Using the brain-tumour incidence in 1999 as baseline, they calculated the incidence in the consecutive years assuming that use of mobile phones was associated with a 10-year latency RR for brain tumours of: 1.0, 1.2, 1.5 or 2.0. Visually comparing the predicted and observed incidence trends the authors concluded that the RR=1.5 scenario showed a similar trend to the observed trend for glioma.

The authors conclude that there was no association between number of mobile phone subscriptions and brain tumour incidence in Korea. The decreasing proportion of tumours classified as of unspecified topo- or morphology and corresponding increase of specific tumours groups has also been observed in previous studies and does not suggest mobile phones as a primary risk factor for brain tumours. The evaluation of mobile phone risk scenarios is hampered by lack of formal testing as well as lack of age and gender data on mobile phone usage meaning that all participants had the same probability of being a mobile phone user. Factors speaking against a causal association include that the increase in glioma corresponded to a decrease in unspecified tumours and the observed increasing incidence of glioma appears to start before the predicted incidence in the RR=1.5 scenario.

Since brain, parotid gland, thyroid, and colorectal cancer have been hypothesized to be linked to RF-EMF exposure, de Vocht et al. [69] evaluated the time trends of the incidence of these tumours by sex and 5-year age groups for 1996 to 2017 for England using data from the UK Office for National Statistics. Over the whole time period, brain tumour incidence was found to steadily increase (+19% for women and +18% for men) without evidence of a (statistically significant) change point. The same pattern was found for parotid glands: a steady increase without any change point was observed. Tumours of the thyroid gland have markedly increased, in particular in women where incidence has more than tripled from 1996 to 2017 but no indication of a change point was found. The incidence rates of colorectal cancer have been relatively stable for women (+3% from 1999 to 2017) and were somewhat increasing for men (+15% from 1999 to 2017). The author concluded that in agreement with data from other countries, there is little evidence of an association between mobile phone use and brain or parotid gland cancer, while the hypotheses of associations with thyroid or colorectal cancer are similarly weak.

This time trend study shares the same strengths and limitations of other time trend studies. The absence of change points in the trends argues against an association between mobile phone and the risk of these tumours. A large part of the population started to use mobile phones around the year 2000. Thus, if there were a risk, increase in incidence would be mostly occur about 5 to 15 years later with a corresponding change point in the trends. Steady increases may be due to improved diagnostic procedures, increases in obesity (a risk factor for thyroid cancer), ageing of the population and more complete cancer registration over time.

4.1.3 Reproduction

Yu et al. [70] performed a systematic review and meta-analysis on mobile phone use/ exposure and sperm quality. Observational human studies (n=5; all of cross-sectional design), animal experimental studies (n=26) and in vitro studies on human sperm (n=8) were included. Sperm density, motility, viability and morphology were evaluated. Human observational studies did not indicate associations of mobile phone use and sperm quality. In vitro studies on RF-EMF exposure suggested reduced motility and viability of exposed human sperm. Sperm motility and viability was also reduced in exposed rats.

It is unclear how mobile phone use in men was associated with RF-EMF exposure of the testes. The authors also note that several studies were of low quality, which may have introduced heterogeneity between studies and hampered interpretation. Exposure set-ups in in vitro and in vivo studies did mostly not control for thermal effects. Overall, results indicate that possible RF-EMF effects on sperm quality require follow up with more standardized study protocols and improved exposure assessment methods.

Kim et al. [71] performed a systematic review and meta-analysis of studies published since 2012 on mobile phone use and semen motility, viability and concentration. They included nine in vivo and nine in vitro studies amounting to a total of 4280 semen samples. The mean difference between exposed and unexposed was -5.98% (95%CI: -9.85 to -2.10, 16 studies/3446 samples) for motility (16 studies, 3446 samples), -11.47% (95%CI: -18.8 to -4.07, 6 studies/ 783) for viability and -5.94 $*10^6$/mL (95%CI: -10.41 to -1.48, 12 studies/3796 samples) for concentration. The lower semen parameter scores for samples with higher exposure were generally also observed in analyses restricted to in vivo/epidemiological or in vitro studies, and both in studies where the control group were non-exposed and where the control group were low exposed. Only two epidemiological studies addressed whether the phone was stored in the trouser pocket i.e. near the testis. The combined estimates for these studies did not show significant differences in semen parameters between exposure groups. The authors conclude that mobile phone use decreases overall sperm quality. They also note that existing studies often rely on self-reported retrospective usage data and recommend that future studies be prospective.

A meta-analysis is restricted by the quality of available studies and as has been previously noted in SSM, many studies on semen quality may have been affected by participation bias and have had inadequate exposure assessment regarding testicular exposure. In addition, potential lifestyle confounders that may be associated with mobile phone usage are rarely addressed in detail and exposure set-ups in the experimental studies are mostly not preventing confounding from heat exposure. As noted also for the above study by Yu et al, studies with better exposure assessment and preferably comparable study protocols are required to answer the question of whether RF-EMF affects semen quality.

Maric et al. [72] performed a systematic search of publications addressing environmental exposures and male infertility. Regarding electromagnetic field exposure, the authors state that continuous development of technology and application represent a challenge to conduct comparable studies, and that it was difficult to clearly identify causality between specific exposure types and their associated biological effects.

While the authors report performing systematic searches of the literature, only 6 studies were included in this review, of which 2 addressed ELF-MF exposures and others RF-EMF exposure. Studies were not meta-analysed but just summarized. As such it remains unclear how to interpret the results, if studies reported heterogenous results and what underlying issues could be.

Maluin et al. [73] concluded in their systematic review that RF-EMF emitted by mobile phones and Wi-Fi devices can cause testosterone reduction, whereas the effect on gonadotrophic hormones (follicle-stimulating hormone and luteinizing hormone) was found to be inconclusive.

The review only included three studies performed in humans. Of these, the first study was a very small experimental study in just 20 participants. The second study was performed among men attending a fertility clinic, but results were not adjusted for any potential confounders, not even age. The third study included 146 participants, but again did not adjust for any confounders. Overall, summarising studies with insufficient quality will result in uninformative reviews, even if done in a systematic way.

In order to investigate the impact of RF-EMF exposure on fertility and sperm quality, Hatch et al. [74] analyzed data from two prospective preconception cohort studies conducted between 2012 and 2020 in Denmark and North America. About 3,000 men were asked whether and where they carried a mobile phone for how long on their body. Time to pregnancy in the female partners was assessed using bi-monthly follow-up questionnaires up to a maximum of 12 months or until reported conception. Overall, there was little evidence that carrying a mobile phone in a front trouser pocket affected male fertility. Only in underweight and normal-weight men (body mass index (BMI) <25 kg/m^2) carrying a mobile phone in the front trouser pocket was associated with a longer time period until successful pregnancy of their partners. However, an exposure-response relationship was not evident with respect to how long the mobile phone was carried in the front trouser pocket. Analysis of almost 800 sperm samples in a subgroup of these cohorts showed no effect of the mobile phone in the front trouser pocket on sperm quality (volume, concentration, and motility). The study accounted for a number of potential confounding variables: Ethnicity, education, male and female BMI, household income, frequency of sexual intercourse, and female age. For men, the study also took into account smoking, sleep, work, age, history of sexually transmitted diseases, physical activity, and consumption of sugar-sweetened drinks as potential confounders. Exposure assessment was done prospectively.

This study is substantially more informative than previous epidemiological studies on sperm quality. The association with fertility found in men with relatively low or normal BMI could be a chance finding. However, the authors also speculate that in relatively slim men, the distance between mobile phone and testes is small, and their testes are thus more RF-EMF exposed than those of other study participants. A considerable weakness is that there are to date no data regarding quantification of RF-EMF exposure of the reproductive organs from carrying a mobile phone in the front trouser pocket. Until such data are available, it would not be possible to conclude whether any observed association are due to radiation or other effects of carrying the phone in the pocket. It should also be noted that the study participants tested the sperm quality with a self-test at home. It is unclear whether this had a negative impact on data quality.

4.1.4 Self-reported electromagnetic hypersensitivity (EHS) and symptoms

A total of 1,019 volunteer students aged 18–24 years at the Niğde Ömer Halisdemir and Recep Tayip Erdoğan Universities answered a questionnaire on mobile phone use and non-specific symptoms of ill health in 2018 and 2019 (İkinci Keleş, 2021 [75]). Most students reported to spend 4–8 hours per day on their cell phones. According to Chi-square tests, headache, concentration deficits, tiredness on waking in the morning, hyperactivity, general feeling of fatigue, and lethargy were statistically positively correlated with duration of mobile phone use.

In this cross-sectional analysis no confounders were considered, and thus it remains open whether observed associations were due to other factors related to excessive mobile phone use. Further, it is not clear from the paper how mobile use was defined and whether it includes activities such browsing the internet or interacting with social media. If the latter, exposure may rather be a proxy for screen time but not for RF-EMF exposure. Little information about the recruitment process is given and the participation rate is not reported.

Tyagi et al. [76] conducted a survey in India about the impact of the Covid-19 lockdown on excessive mobile phone use, which was distributed by e-mail, Whatsapp, and Facebook to the contacts of the investigators. In 90% of the 122 respondents, an increase in the usage of mobile phone technology during lockdown was observed, and 95% of the participants perceived an increased risk of developing certain health problems due to excessive mobile phones use. The authors found that participants in the age group 15–30 years were more strongly affected and suggest that strategies should be planned to decrease the psychological and physiological effects of the overuse of technology during lockdown due to pandemics. This study did not study health effects from RF-EMF but rather addressed self-reports of mobile phone usage, and self-reports of symptoms and risk perception. The sample is very small and selective and thus they may not be representative for the whole population, although the findings are in line with anecdotal reports.

The study of Chongchitpaisan et al. [77] aimed to investigate the effect of modern communication technology use on triggering migraine headaches in high school students in Chiang Mai Province by means of a prospective time series study. The study included 145 smartphone users who each completed a headache diary for two to four months resulting in a total of 12,969 data entries. The output power of the own phone was measured and recorded by a smartphone application. In Generalized Estimating Equation analysis, adjusted for age, anxiety score, internet use, and hands-free use, associations between mobile phone output power and the likelihood of a migraine attack was observed. Notably, the mobile phone output power six days prior to the migraine attack was positively correlated with the migraine risk, whereas high output power at the day of the diary entry was associated with a lower risk for an attack.

This study follows an interesting approach to study whether RF-EMF triggers migraine attacks. It is not described how the app was able to measure the output power of the phones and no validation for these data has been provided. The results are inconsistent in terms of direction of associations for various EMF exposure surrogates. It is unclear, why results are presented for lag 0 and 6 but not for the days between. Possibly the app data are not reliable or the statistical model was over-specified with many lag effects. It seems rather unlikely that migraine attacks were triggered because of RF-EMF exposure six days before, followed by a protective effect from current day exposure. The latter may be the result of reverse causation. Migraine symptoms may prevent from using the mobile phone.

Cabre-Riera et al. [78] combined a Dutch and a Spanish birth-cohort, to investigate association of RF exposure and sleep in children. The original cohort size at enrolment during pregnancy, was 11,316. For the present study, 1842 children aged 9-12 were included. For 1159 children, all-day data on use of mobile communication devices and sleep disturbances were collected by questionnaire. For 1080 children a wrist accelerometer was used to gather objective sleep data for a 7-day period and a subsample of these children (n=335) also provided daily information on use of mobile communication between 7 pm and falling asleep for each of these seven days. RF-EMF exposure was estimated from an integrated model combining information on individual size and weight, usage patterns for different devices and their output power. Information was aggregated as all day whole brain total RF-EMF dose and total and source specific evening whole brain dose. Information on a range of potential confounders was also obtained from questionnaires. Missing confounder values were imputed. The children spent on average 2.5 min/day talking on a mobile phone and this was the main contributor (78%) to all day whole brain dose (median: 60 mJ/kg/day). In cross-sectional analyses, based on questionnaire data, the only observed associations were between overall daily dose from screen-activities and excessive somnolence and longer sleep onset latency. In longitudinal analyses based on diary and accelerometer data, exposure from screen activities was not associated with sleep parameters, whereas high dose (>2.3 mJ/kg/evening) from phone calls was associated with shorter sleep duration and longer sleep onset latency. After correcting for multiple testing only the association between evening time exposure from phone calls and sleep duration persisted, with shorter sleep duration in high compared to low exposed preadolescents (-11.9 min/, 95%-21.2- -2.5). The authors stress that their data do not allow them to determine if the association was due to the RF-exposure or other factors such as mental arousal or sleep displacement. The authors conclude that all-day RF-EMF dose was not associated with sleep and suggest that evening exposure may a relevant time-window for studies of association with sleep.

In spite of the comparatively large population, only 335 children contributed to the longitudinal analysis which could increase the risk of chance findings, and as the authors point out it is not possible to determine if the observed association were due to RF-exposure or other factors. While it is commendable to try to integrate all exposures and quantify exposure of specific body parts, the elaborate exposure algorithm includes a large number of assumptions, and some exposure misclassification is inevitable and may have driven results towards the null.

4.1.5 Other outcomes

Kacprzyk et al. [79] conducted a systematic review on mobile phone use and tinnitus. They included two cohort studies, one case–control study, and three cross-sectional studies in their meta-analysis. They concluded that current scientific knowledge, including high-quality studies with a reliable exposure assessment based on network operator data, does not support the hypothesis that mobile phone use is associated with the risk to develop a tinnitus.

Taziki Balajelini et al. [80] systematically reviewed and meta-analysed five studies on hearing impairment, meta-analysing outcomes such as tinnitus, hearing loss and vestibular schwannoma together. Overall, no increased risks were observed.
It is unclear how the authors dealt with different exposure levels that were evaluated in the original studies. Interpretation of the overall results is hampered by the fact that diverging outcomes were analysed together.

In a cross-sectional study, Ghandehari et al (2021) investigated micronuclei in the oral mucosa of 100 mobile phone users selected among patients at the Dentistry Faculty of Islamic Azad University. Participants were required to live in Tehran, use a mobile phone in non-hands-free mode, and not be occupationally exposed to chemicals, not have a history of systemic disease, radiotherapy, smoking, use of alcohol or medication. Furthermore, they were required to not have had recent viral infection or oral lesions. Micronuclei were identified from mucosal samples from the cheek where the phone was predominantly used. For each participant, 500 cells were examined, In Pearson correlation analysis, a significant correlation was observed between self-reported daily hours of mobile phone use and number of cells with micronuclei (r=0.70) and number of micronuclei per cell (r=0.75). Age and sex were evaluated as covariates, but not included in final analysis. The authors conclude that amount of mobile phone use was associated with frequency of micronuclei.

Concerns include that the counting of micronuclei is not stated as being blinded to exposure status and the number of cells examined per subject is low. It has been recommended to score 2000 or more cells per sample [81]. Further, potential confounding from factors such as diet were not considered. In addition, a comparison of the most and least exposed cheeks might have been informative but was not presented. It should also be noted that even if the association may be true, it cannot be concluded if it is due to RF-radiation, heat or other effects of holding a phone to the head.

Khalil et al. [82] performed a cross sectional study of 100 volunteer students from Yarmouk university, Jordan. Participants were required to be free from chronic systemic disease, previous head and neck injury, history of chemo- or radiotherapy as well oral lesions or signs of infections or inflammation. The inflammatory markers immunoglobin A and myeloperoxidase were measured in saliva samples. Use of mobile phones was assessed by questionnaire and classified into three exposure categories, based on either years of mobile phone use or daily call time. ANOVA analysis showed no significant differences between inflammatory marker levels in the different exposure groups.

Apart from the small size of the study, limitations which may have impaired the ability to detect an association are the crude exposure assessment, and not accounting for modes of use in classifying exposure, especially since 89% of participants reported “awareness of phone side effects”. Earphones were used frequently, which strongly reduced exposure to the head when calling as compared to calling while holding the phone to the head.

Dongus et al. [83] reviewed biological or health effects of Wi-Fi radiation. They performed a systematic literature search and after applying strict selection criteria identified 23 studies. Included studies fulfilled basic methodological quality criteria, such as having meaningful exposure assessment, account for potential selection bias and confounding and were relevant, were conducted as a blinded experiments. As the number of studies per outcome was small, they abstained from meta-analyses. They concluded that there was no consistent association with any endpoint and that the few positive associations reported were not consistent between studies or did not exhibit positive exposure-response relationships. They recommended that future studies explore different aspects of RF-EMF exposure such as frequency, signal characteristics as well as intensity and duration of exposure. As they had to exclude a majority of the identified studies due to limitations in design or reporting of the study, they highlighted the importance of adhering to high standards in the interest of furthering knowledge in the field.

4.1.6 Conclusions epidemiological studies

Several new epidemiological studies did not find an association between mobile phone use and brain tumours. So far, research in children has been scarce. The international MOBI-kids case-control study is by far the largest study on this topic so far. Overall, the study found a trend of decreased risk with increasing mobile phone use. However, in-depth analyses suggest that most likely this observation is biased due to proxy interviews and prodromal symptoms. This illustrates that case-controls studies may have limited potential to detect small risks since they are prone to various types of biases. An alternative approach is to analyse temporal trends in brain tumour incidence and compare with increases in mobile phone subscriptions. Several such papers have been published on various types of tumours in the head region, which do not indicate an increase in adults paralleled to the uptake of mobile phone use.

A number of systematic reviews postulate an association between mobile phone use and semen quality. However, these reviews are based on low quality study vulnerable to selection bias and using crude and retrospective exposure assessment. Strikingly, a paper evaluating data from two large prospective cohort studies found little evidence that carrying a mobile phone in a front trouser pocket affected male fertility, although a significant effect in men with a BMI of less than 25 kg/m² deserves follow-up investigation. In the same study, position of the phone on the body was not associated with various aspects of semen quality. For other outcomes, little progress has been made in the period relevant for this report.

4.2 Human studies

In 2021 four human experimental studies on RF-EMF effects were published, all investigating effects on brain activity. Two used electroencephalography (EEG, Wallace et al. [84], Dalecki et al. 2021 [85]), one magnetencephalography (MEG; Wallace et al. [86]) and one functional magnet resonance imaging (fMRI; Yang et al. 2021 [87]).

In view of the accumulating evidence showing that the effect of RF-EMF exposure on the resting state wake EEG, in particular on the alpha frequency band, is less consistent than previously reported two studies addressed reasons for inconsistent results.

A study by Wallace et al. (2021a) explored the impact of the definition of alpha frequency band range (fixed frequency range: 8-12 Hz vs individually assessed alpha frequency range) on the results. The resting state EEG was recorded in 21 healthy volunteers (10 females, 11 males, mean age ± SD: 25.1 ± 3.6 years) in an eyes-open (EO) and an eyes-closed (EC) condition. To reduce the variability in the outcome parameters subjects with a history of head injury, neurological or psychiatric disease or any chronic disease, disability or recent acute illness during last month as well as pregnant women were excluded. Furthermore, only subjects with regular sleep habits, no medication, no smoking and no-drug use (confirmed by multiple urine drug test) were included. During the study participants had to abstain from consuming alcohol and caffeine for 24 hours before each experimental session. They had to maintain the regular sleep-wake cycle and not to use their mobile phone on the days of the experiment. Participants had been fasting for at least two and half hours before the recordings. To avoid potential confounding effects from hormonal levels during the menstrual cycle, all women were tested in the follicular phase of their cycle. In a double-blind, randomised and counterbalanced crossover design, subjects were exposed at the left ear to either sham or a pulse modulated (217 Hz) 900 MHz GSM signal with a mean power of 250 mW and a peak power of 2 W (max SAR_{1g} = 0.70 W/kg). Sessions were scheduled one week apart. Time of the day was controlled within subjects. Each of the two sessions started with a 12 min EEG baseline recording in parallel to a MEG recording (runs 1 and 2), followed by 12 min EEG baseline recording without MEG (runs 3 and 4). Both baseline recordings were without RF-EMF exposure. The baseline recordings were immediately followed by a recording under exposure (sham or GSM) for a duration of 25 min and 30 seconds (runs 5 to 8). A 25 min 30 s EEG recording in parallel to MEG recording (runs 9 to 12) without RF-EMF exposure finished the session. All runs included a 3 min EO and a 3 min EC recording condition. EEG was recorded with a cap from 74 channels, four electrodes close to the ear-slits of the cap were excluded from analysis. Peak maximum alpha frequency (PAF) was individually computed from 13 parietal and occipital electrodes based on the runs 3 and 4 of the baseline recording averaged over both sessions. The individual alpha frequency range (IAF) was defined as the interval PAF ± 2 Hz. Baseline corrected exposure values were obtained for each electrode from log-transformed values separately. Separately for the IAF, which was 7.78 – 11.78 Hz averaged over subjects, and the fixed alpha frequency range 8-12 Hz a three-way repeated measures ANOVA with factors recording (EO vs EC) and period (baseline vs exposure) and exposure (sham vs real exposure) was applied. The analyses showed a statistically significant period (centro-parietal, temoro-parietal, parietal, occipito-parietal and occipital electrodes) and eyes (over the whole scalp) condition effects for both the IAF and the 8-12 Hz frequency range. The baseline-corrected EEG-data were submitted to a one-way repeated measures ANOVA with exposure (sham and GSM) as factors. In the EO condition, higher values were observed under exposure as compared to sham at pre-frontal, frontal, parietal, parietal-occipital and occipital electrode site for the 8-12 Hz frequency band. The differences between exposure conditions, however, were not statistically significant. In the EC condition, baseline-corrected power values for the 8-12 Hz frequency range were lower under exposure as compared to sham in the EC condition, mainly at frontal and parieto-occipital sites. Again, the differences did not reach statistical significance. The results for the IAF frequency range were very similar. None of the differences between exposure conditions was statistically significant. The results underline first that irrespective of the eyes condition (open vs. closed) there is no RF-EMF effect on the alpha rhythm in the wake EEG and second that there is no different sensitivity to MP exposure related to inter-individual variability of the alpha rhythm. These results underline that effects on the EEG in the alpha frequency range are less consistent than previously reported as for example in the Appendic B of the ICNIRP Guidelines for Limiting Exposure to Electromagnetic Fields (100 kHz to 300 GHz) (ICNIRP 2020, [88]). For a more detailed discussion see also Wallace and Selmaoui [89] and Danker-Hopfe et al. [90].

Dalecki et al. (2021) who also aimed at investigating possible reasons for inconsistent results with RF-EMF effects of the resting wake EEG also looked at the recording condition, i.e. recording with eyes closed (EC) and eyes open (EO). They furthermore investigated whether the duration of exposure could be a factor that explains deviating results. Thirty-six healthy subjects (18 males and 18 females, mean ± SD: 24.4 ± 6.3 years) participated in this double-blind, randomized study that was fully counterbalanced between participants and within gender. In this study participants were excluded if they had any history of neurological disorder, head trauma, or were taking medication that acts on the central nervous system. Participants were instructed to abstain from caffeinated foods and drinks within 1 h, and from mobile phone use within 2 h prior to their arrival at the laboratory. This was verified via self-report. Subjects were exposed to three different exposure conditions: sham, low and high RF-EMF with peak spatial specific absorption rates of SAR_{10g} = 0, 1, and 2 W/kg, respectively. A simulated 920 MHz signal was administered to the left hemisphere using a sXh920 planar exposure system. For each participant four visits to the laboratory were scheduled at least seven days apart at the same time of the day. Each exposure session started with a 16 min baseline block without any exposure, during which resting EEG was recorded for 1 min 40s with eyes open followed by 1 min and 40s recording with eyes closed. The baseline block was followed by a 30 min exposure block during which one of the three exposure conditions was administered. The session ended with another 30 min block without exposure. These two blocks began with a resting EEG recording (EO: 1 min 40s followed by EC: 1 min 40s) and ended with a second recording (same conditions and same duration) starting 26 min after the start of each block. Thus, overall, the duration of EEG recording was comparatively short. Although EEG was recorded from 19 scalp channels placed according to the international 10/20 system, only nine electrodes grouped into three regions (Frontal: Fz, F3, F4; Central: Cz, C3, C4; Parietal: Pz, P3, and P4) were used for statistical analysis. The dependent variable was the change of power in the alpha frequency range (8-12 Hz) from baseline to the EEG recorded under exposure. To standardize for EEG changes over time. i.e. with the level of alertness, the EEG alpha power values were converted to z-scores for each recording situation, i.e. separately for the baseline recording and each of the recordings under exposure (at the beginning and at the end). The hypothesis that alpha power was dependent on eye condition (EC or EO) was tested by a 3 (exposure: sham, low, high) x 3 (sagittality: frontal, central parietal) x 2 (laterality: left, right) x 2 (EC, EO) repeated measures ANOVA, which was performed with one-tailed planned contrasts. Figures with results of raw data are presented but not further analysed. Results showed that there was a greater increase in EEG alpha power in the eyes open than in the eyes closed condition in both the low and the high RF-EMF condition as compared to sham. This observation is partly in contrast to Wallace et al. 2021 who observed a non-statistically significant increase in alpha power under exposure in the EO condition. However, in the EC condition Wallace et al. 2021 observed a decrease of alpha power under exposure as compared to sham, which was again not statistically significant. With regard to exposure duration, an increase in alpha power from the beginning to the end of the exposure block was observed in the EO condition under high exposure. The change in the low exposure condition was not statistically significant and results for the EC condition are not reported. The authors conclude that their results *suggest that the use of eyes closed conditions, and insufficient RF-EMF exposure durations are likely explanations for the failure of some studies to detect an RF-EMF exposure related increase in alpha power* (Dalecki et al. 2021, p.317). However, another explanation for heterogeneous results with respects to significant exposure effects could be the statistical approach. While most studies use two-tailed p-values to evaluate statistical significance, Dalecki et al. (2021) use one-tailed p-values.

To further pursue the problem of RF-EMF effects on the waking alpha band activity Wallace et al. (2021b) performed a magnetencephalography (MEG) study. MEG like EEG records cortical activity with a high temporal resolution. MEG, however, enables a higher spatial accuracy not only in surface recording but also in source localization. To control for various confounders, several parameters were additionally assessed: heart rate and heart rate variability (HRV) to control for autonomous nervous system activity, salivary cortisol as indicator of the hypothalamic-pituitary-adrenal (HPA) axis, and chromogranin A as well as alpha amylase as indicators of the sympatho-adreno-meduallary system. Furthermore, the caffeine concentration was assessed. Thirty-two right-handed volunteers (15 males and 17 females, mean ± SD: 24.8 ± 3.5 years) were recruited following the same screening criteria as described above (Wallace et al. 2021a). Again, women were only investigated in the follicular phase of their menstrual cycle. In a double-blind randomized and counterbalanced cross-over design subjects were exposed to either sham or a GSM signal as described in Wallace et al. (2021a). At the same time of the day two experimental sessions were scheduled one week apart. MEG was recorded prior to and following a 25min 30s exposure. Due to electromagnetic interferences between the RF-EMF field and the MEG sensors, MEG data were not analysed during exposure. Exposure condition and the experimental protocol were the same as described in Wallace et al (2021a). The maximum SAR values averaged over 10g tissue, 1 g tissue and the peak were measured at 0.49 W/kg, 0.70 W/kg and 0.93 W/kg, respectively. Continuous MEG cortical signals were collected using a whole-head MEG system with 102 magnetometers and 204 planar gradiometers. A three-way repeated measures ANOVA with factors time (pre-post exposure), eyes condition (open, closed), and exposure conditions (sham, GSM exposure) revealed statistically significant effects for all three factors. Results indicated a significant effect of 900 MHz RF-EMF exposure on the alpha band MEG activity, mainly represented as a decrease of spectral power amplitudes. The eyes open sensor analysis revealed a modification of the entire alpha band and both frequency sub-bands after RF-EMF exposure, especially at the parietal cortex. These results were confirmed by analysis at source level for the fronto-parietal region. For the EC condition the sensor space analysis showed a decrease of the entire alpha band power and the upper alpha power after RF-EMF exposure, especially at the temporo-occipital region. These results were also supported by source space analysis. Overall, this MEG study showed a widespread decrease in the alpha band power of the resting spontaneous EEG. The affected regions vary depending on the eyes open and eyes closed condition. Finally, none of the control parameters showed statistically significant differences between the GSM and the sham exposure condition.

Effects of LTE exposure on functional connectivity and network properties were investigated by Yang et al. (2021) in a double-blind parallel group design using functional magnetic imaging. Thirty-four subjects (19 males and 15 females) in the age range between 19 and 26 years (mean ±SD: 23.6 ± 4.8 years) were equally divided into two groups, one receiving sham (10 males and 7 females) and the other LTE exposure (9 males and 8 females). It is not reported whether the assignment to groups followed a random procedure. From the description of participant recruitment, it seems that subjects were recruited from a patient population: ”*The researchers selected the candidates with the history of neurological and psychiatric disorders”* (Yang et al.2021, page 5756). If so, it is not reported whether there were differences in the distribution of diagnoses between groups. The experimental session consisted of a structural and a functional MRI performed by a 3.0 T scanner followed by a 30 min sham or LTE exposure, respectively. The session ended with another functional fMRI, which started approximately 1 min after the end of exposure. The LTE signal was generated by a 2.573 GHz generated an amplified by an RF power amplifier, which was further delivered to a standard dipole. The power was adjusted to 24 dBm (mean value), which is the theoretical maximal emission by an LTE terminal. SAR_{10g} and SAR_{1g} values were calculated at an individual level. The $pSAR_{10g}$ was 0.61 ± 0.14 W/kg, with a maximal value of 1.02 W/kg, while $pSAR_{1g}$ was 2.47 ± 0.65 W/kg with a maximal value of 4.08 W/kg. Within-session and between-session comparisons were performed for functional connectivity and network properties. The results indicated that acute LTE exposure beneath the safety limits modulated both the functional connection and graph-based properties. The latter models the brain as a complex network represented graphically by a collection of nodes and edges. The authors do not provide information on group differences in functional connectivity (strength and diversity) in the pre-exposure assessments. Correlation analysis of individual SAR levels and functional as well as graph-based outcomes showed that functional connectivity (strength and diversity) was not correlated with peak SAR values, which demonstrated that SAR values might not be the right metric to quantify the neurophysiological effects of EMF exposure. A limitation of the study certainly is that the vigilance level of the subjects was not controlled. Subjects were asked whether they fell asleep during the scan and although none of the subjects reported to have fallen asleep, it cannot completely be ruled out that results are affected by this factor.

4.2.1 Conclusion human studies

Results concerning RF-EMF effects on the resting state EEG in the alpha frequency range continue to be inconsistent even in studies of high quality. Results published in 2021 showed either no effect (EEG study by Wallace et al. 2021a), an increase in alpha (EEG study by Dalecki et al, 2021), or a decrease in alpha power (MEG study by Wallace et al. 2021b). The search for reasons explaining the inconsistency indicate that results do not seem to depend on the definition of the alpha band frequency range, i.e. analyses for a fixed frequency range of 8-12 Hz yield the same results as analyses based on individual peak alpha frequency ranges. It seems that results vary with the eyes condition. While an EEG study found that the use of the eyes closed condition probably contributes to not observing RF-EMF effects, an MEG study observed that differences between exposure conditions seems to be more pronounced in the eyes closed condition. Since differences between exposure conditions are observed in both directions (increases and decreases) it is suggested not to use one-tailed statistical tests. Finally in a parallel-group design study effects of RF-EMF exposure on functional connectivity and network properties have been reported. This study, however, indicated that SAR values do not seem to be the right metric to quantify neurophysiological effects. In MRI studies, however, it must be kept in mind that the method of assessment of exposure effects itself represents a complex EMF exposure situation during scanning. These exposure conditions are the same in the sham and the exposure condition related to the experimental signal.

Table 4.2.1: Human studies on exposure to RF fields

Endpoints	Reference	Exposure condition	Sample	Results
Functional connectivity	Yang et al. (2021)	Sham exposure and LTE (2.573 GHz) exposure, $pSAR_{10g}$ = 0.61 ± 0.14 W/kg, max: 1.02 W/kg, $pSAR_{1g}$ = 2.47 ± 0.65 W/kg, max: 4.08 W/kg,	34 subjects (19 males, 15 females) mean ± SD: 23.6 ± 4.8 years sham group: 17 subjects (10 males and 7 females), exposure group: 17 subjects (9 males and 8 females)	LTE exposure beneath the safety limits modulated both the functional connection and graph-based properties. SAR averaged over a certain tissue mass was not an appropriate metric to characterize the effect of functional activity,
Waking state EEG	Wallace et al. (2021a)	Pulse modulated (217 Hz) 900 MHz GSM signal, mean power 250 mW, peak power 2W; max SAR_{1g} = 0.70 W/kg and sham exposure	21 heathy subjects (10 males, 11 females), mean ± SD: 25.1 ± 3.6 years	RF-EMF exposure did not modulate alpha band EEG activity in the resting state
	Dalecki et al. (2021)	Sham exposure, low RF-EMF exposure: 920 MHz SAR_{10g} = 0.1 W/kg; high RF-EMF exposure SAR_{10g} = 2 W/kg	36 healthy subjects (18 males, 18 females), mean ± SD: 24.4 ± 6.3 years	Alpha power increase was found to be greater for the eyes open than eyes closed EEG during both the high (P=0.04) andlLow (P=0.04) RF-EMF exposures.

	Wallace et al. (2021b)	Pulse modulated (217 Hz) 900 MHz GSM signal, mean power 250 mW, peak power 2W; max SAR_{10g} = 0.49 W/kg, max SAR_{1g} = 0.70 W/kg, peak SAR_{1g} = 0.93 W/kg and sham exposure	32 healthy subjects (15 males, 17 females), mean ± SD: 24.8 ± 3.5 years	Widespread decrease in the alpha band power of the resting spontaneous EEG. The affected regions vary depending on the eyes open and eyes closed condition. eyes condition

4.3 Animal Studies

This year, as in previous years, a variety of endpoints was investigated, including effects on the brain and on behaviour, genotoxicity, male fertility, development, oxidative stress and temperature changes.

4.3.1 Effects on brain

Lin et al. [91] exposed male ICR mice (n=12 per group) to L-band (~2 GHz) microwaves at 0.5, 1.0, and 1.5 W/m^2 for 1 h per day during 4 or 8 weeks and examined the brain function at different time periods after exposure. Exposure during 4 weeks at 1.5 W/m^2 caused increased apoptosis in the hippocampus (CA1 and CA3) and cerebral cortex (the first somatosensory cortex). After 8 weeks exposure this was found with all exposure levels. No changes were observed in c-fos levels, but after 8 weeks exposure at 1.5 W/m^2 an increased level of acetylcholinesterase was found. Also, after 8 weeks exposure, but with both 1.0 and 1.5 W/m^2, increased oxidative stress was observed.

Othman et al. [92] exposed female Sprague Dawley rats (n=9 per group) to 2.45 GHz fields for 30 min once or daily, 5 days per week for 10 days. The brain SAR varied from 0.069 to 0.075 W/kg. Animals were euthanised 1.5 h after the last exposure (with the single and repeated exposures) or 24 h after the single exposure. A progressive decrease in glucocorticoid receptors and heat shock protein HSP-70 was observed after acute and repeated irradiation in the somatosensory cortex, hypothalamus and hippocampus. In the limbic cortex, values for both biomarkers increased after repeated exposure. Glial fibrillary acidic protein (GFAP) values in all brain regions studied was not changed by exposure.

He et al. [93] exposed male BALB/c mice (group size not provided) to 2.45 GHz pulsed fields (500 pps) for 20 min per day during 2, 4, or 6 weeks. The average SAR was 6 W/kg. Exposure significantly decreased the number of proliferating and differentiating cells in the dentate gyrus of the hippocampus, resulting in a reduced neurogenesis rate. Moreover, alterations in the phenotypes of activated microglia and decreased expression levels of microglial CX3C chemokine receptor 1 (CX3CR1), but not sirtuin 1 (SIRT1), were observed in the brains of exposed mice. Loss of CX3CR1 signalling is the critical event that leads to an increase in adult hippocampal neurogenesis. SIRT1 is an enzyme associated with the cellular response to inflammatory, metabolic, and oxidative stressors.

Wang et al. [94] exposed the fruit fly *Drosophila melanogaster* to 3.5 GHz fields at 0.1, 1, and 10 W/m^2 continously during 3 days. In the second part of the study, flies were allowed to mate during the exposure and the progeny was exposed for an unmentioned time period. Short-term exposure increased the activity level and reduced the sleep duration in the flies, while long-term exposure reduced the activity level and increased the sleep duration of F1 male flies. Also after long-term exposure, the expression of several genes related with heat stress responses was increased, the expression of several genes associated with the circadian clock was altered, but without a clear exposure-related pattern. The activity of several neurotransmitters associated with sleep was decreased and the expression levels of several associated genes were altered, but again without a clear exposure-related pattern.

4.3.2 Cognition and behaviour

Bayat et al. [95] exposed male Sprague Dawley rats (n=6-10 per group) to 2.4 GHz WiFi fields for 2 h per day during 45 days. Vascular dementia was induced by bilateral-common carotid occlusion. Because the animals could move freely during exposure, the power density varied from 0.0032 to 0.018 to mW/cm^2 (0.032 to 0.18 W/m^2) and the SAR from 0.0060 to 0.0346 W/kg, but no dosimetry is reported. The induced vascular dementia led to impairment in spatial learning and memory that were associated with impairment of long-term potentiation (LTP), decrease of basal-synaptic transmission, increase of GABA transmission, and with decline of the release of neurotransmitters and hippocampal cell loss. Wi-Fi exposure significantly recovered the learning and memory performance, LTP induction, and cell loss without any effect on synaptic transmission.

Rui et al. [96] investigated the effect of exposure to 5.8 GHz for 2 or 4 h at a brain SAR of 2.33 W/kg on the learning and memory ability of rats. They used male Sprague Dawley rats (n=25 per group) and exposed them for 2 or 4 h per day during 15 days. Neither exposure regime resulted in changes in memory and learning capability, nor in changes in the morphology of the brain tissue, or in the level of several factors associated with neuron function and plasticity (PSD95, synaptophysin, p-CREB and CREB) in the hippocampus. There is no description of the dosimetry.

Delen et al. [97] exposed male Wistar rats (n=6 per group) to 2600 MHz fields for 30 min per day, 5 days per week during 30 days. The SAR_{10g} in brain was 0.295 W/kg. Exposure resulted in increased oxidative stress, witnessed by a decrease of GSH, GSH-Px and SOD levels and an increase in MPO, MDA, and NOx levels, as well as an increase in structural deformation and apoptosis in brain tissue. Injection with 10 mg/kg/day melatonin for 7 days per week during the 30 days exposure period reduced these effects.

Deniz and Kaplan [98] exposed pregnant Wistar rats to 900 MHz fields for 1 h per day during 21 days (until the end of pregnancy). The electric field in the region of the abdomen was 19.21 mV/m, but the actual exposure of the foetuses was not assessed. Four weeks after birth, male pups (n=6 per group) were used for behavioral tests and subsequently for analysis of the hippocampus. No effects on behaviour were observed, except a small decrease in anxiety on the second of a 2-day test. The total number of pyramidal neurons was decreased in the exposed group. In addition, an increase in both catalase and superoxide dismutase activity in the blood was found, indicating an increase in oxidative stress.

Qubty et al. [99] exposed male and female ICR mice (n= 9-24 per group) to RF fields from an iPhone 7 for 4-5 h per day during 6 weeks, at an electric field level of 20 V/m. They observed no effects on anxiety and object recognition, but a decrease in spatial memory in exposed animals, that was only significant in female mice. There is no description of the dosimetry.

Tan et al. [100] exposed male Wistar rats (n=30 per group) to RF fields for 6 min, at a power density of 10 mW/cm^2 (100 W/m^2). One group was exposed to 2.856 GHz, another to 1.5 GHz and a third to both frequencies consecutively (so they were exposed for 2x6 min). They assessed memory and various enzyme levels 6 h after the exposure. They observed a decrease in spatial memory, changes in EEG, structural injuries, and the downregulation of phosphorylated-Ak strain transforming (p-AKT), phosphorylated-calcium/calmodulin-dependent protein kinase II (p-CaMKII), phosphorylated extracellular signal regulated kinase (p-ERK) and cAMP response element binding (p-CREB), all enzymes associated with memory processes in the brain. Changes in the group that was exposed to both frequencies were generally larger than in the groups receiving exposure to a single frequency.

In a similar design as the previous study, Zhu et al. [101] exposed male Wistar rats (n= 35 per goup) to 1.5 GHz and 4.3 GHz. For 1-28 days after exposure, they analyzed the average escape latency for the Morris water maze task, electroencephalograms, change in hippocampal tissue structure and ultrastructure, content of the Nissl body in the hippocampus, and activities of lactate dehydrogenase and succinate dehydrogenase. All exposed groups showed varying degrees of learning and memory decline and hippocampal structural damage. The largest changes were observed at 7 and 14 days following exposure, and they were larger in the combined exposure group.

Shai et al. [8] exposed male Sprague Dawley rats (n=10 per group) to 1052-1979 MHz fields from a jammer, for 2 h once or for 2 h per day for two weeks. The power density was 86.18 µW/m^2. The single exposure resulted in a decrease in learning time, while the repeated exposures led to a decrease in learning and memory.

4.3.3 Genotoxicity

Gunes et al. [102] used the fruit fly *Drosophila melanogaster* to study the effects of RF fields on genotoxicity. Third instar larvae were exposed with 900, 1800 or 2100 MHz fields for 2, 4 or 6 h for 2 days. The electric fields were 35.2, 41 and 41 V/m, respectively. Genotoxicity was assessed by counting the number of wing mutant clones. An increase in the genotoxicity score was observed for 900 MHz with all three exposure times, for 1800 MHz with 4 h exposure and for 2100 MHz with 4 and 6 h exposure.

4.3.4 Development

Kim et al. [103] exposed pups of ICR mice (n= 11-15 per group) to 1800 MHz fields at a whole-body SAR of 4.0 W/kg for 5 h per day during 4 weeks (from postnatal days 1 to 28). They then evaluated the development of several brain structures in the hippocampus. In the dentate gyrus, the total number of dendritic spines was decreased, but this was not the case in the cornu ammonis. The expression of glutamate receptors and of brain-derived neurotrophic factor (BDNF) was decreased in both the dentate gyrus and cornu ammonis. Memory also decreased in the exposed animals. The authors conclude that these data suggest that hindered neuronal outgrowth following exposure may decrease overall synaptic density during early neurite development of hippocampal neurons.

Wang et al. [94] exposed newly hatched males and females (number not given) of the fruit fly *Drosophila melanogaster* to 3.5 GHz fields at 0.1, 1, and 10 W/m^2 continuously during 3 days. Exposure increased the pupation percentages in the first 3 days and eclosion rate in the first 2 days. In third-instar larvae (n=25 per group) the expression levels of several heat shock protein genes and humoral immune system genes were significantly increased. Also, oxidative stress was increased, and the diversity of the microbiome was decreased. Overall, the rate of development of the flies was increased.

Vargová et al. [104] exposed eggs of the tick *Dermacentor reticulatus* and investigated the development of the larvae (n=200 per group). The exposure was at 900 MHz for 30, 60 or 90 min at a power density of 1 mV/m^2 [they probably mean mW/m^2]. The larvae hatched from eggs exposed for 60 minutes had slightly larger dimensions of all measured body traits compared to the control group, while the other exposed groups were smaller.

Sun et al. [105] exposed cultures of the flatworm *Caenorhabditis elegans* to 9.4 GHz fields modulated at 10, 20 and 50 Hz, for 30 min at an average SARs of 4.33, 8.66 and 21.65 W/kg, respectively. No changes in development, movement, egg production, ROS, and lifespan were detected.

4.3.5 Fertility

Almášiová et al. [106] exposed pregnant female Wistar rats (n= 3 per group) to 2.45 GHz fields for 2 h per day throughout pregnancy at a whole-body SAR of 1.82 W/kg. After birth, male pups (n=6 per group) were left unexposed and their testes examined at an age of 75 days. In the exposed rats, degenerative changes in the testicular parenchyma were observed: irregular seminiferous tubules, degenerated and often desquamated germ cells, significantly decreased diameters of the seminiferous tubules and height of the germinal epithelium, and significantly increased interstitial space. Somatic and germ cells were rich in vacuoles and their organelles often appeared altered. Necrotizing cells were more frequent and empty spaces between Sertoli cells and germ cells were observed. The Leydig cells contained more lipid droplets. Fluoro Jade – C staining for degenerating cells was increased in the exposed animals, and increased superoxide dismutase 2 (SOD2) was found in testicular tissue, indicating increased oxidative stress.

Andrašková et al. [107] used a similar experimental design as the previous study, except that the exposure was at a SAR of 1.73 W/kg, and the testes of the pups were examined at the age of 35 days. They observed similar lightmicroscopic aberrations in the testicular tissue as in the previous study and, using electron microscopy, they observed basement membrane irregularities in seminiferous tubules, vacuolation of the cytoplasm and adversely affected organelles in Sertoli cells, germ cells, Leydig cells, peritubular and endothelial cells. Also the tight junctions between adjacent Sertoli cells were often incomplete, and there was an increase in necrotizing germ cells compared to controls. Fluoro Jade – C staining for degenerating cells was increased in the exposed animals and cell nuclear antigen analysis showed a decline in proliferation of germ cells.

Dong et al. [13] exposed C57BL/6 mice (n=40 per group) to 1.5 GHz fields, 2 x 15 min (the interval between the two exposure periods is not provided) at a whole-body average SAR of 3, 6 or 12 W/kg. At 6 h, 1 day, 3 days, and 7 days after exposure, the pathological structure of the testicles and spermatozoa, as well as serum testosterone and were assessed in groups of 10 animals. Sperm motility parameters were assessed 6 h and 1 day following exposure. No significant pathological or ultrastructural changes were observed in the testicles or spermatozoa, and serum testosterone levels were not changed. Motion of the spermatozoa increased at 6 h after exposure and decreased at 1 day without a clear dependence on exposure level.

Hasan et al. [108] exposed Swiss albino mice (n=10 per group) to 2400 MHz from a mobile phone that resulted in a SAR of 0.087 W/kg, for 40 or 60 min per day during 60 days. Body weight and total erythrocyte count values were significantly decreased, while total leukocyte count, percentage haemoglobin, and serum creatinine values were significantly increased in both exposed groups relative to the sham-exposed control group, with larger changes in the longer exposed group. Histopathological examinations of testicular tissue from the exposed mice showed that this was irregular in shape and non-uniform in size, and contained fewer spermatogenic cells layers, with no clear difference between the two exposure durations. In the kidney, increased interstitial inflammation was observed in the group exposed for 60 min, but not in that exposed for 40 min.

Qin et al. [109] exposed 4-week-old C57Bl/6 J mice (n=12 per group) to 1800 MHz at a whole-body SAR of 0.5 W/kg, for 2 h per day during 21 days, either in the morning or in the evening. Testicular weight, daily sperm productions and testosterone secretion were decreased compared to sham-exposed groups, with a larger effect of the exposures in the morning. They also investigated long non-coding RNAs and identified 615 and 183 differentially expressed LncRNAs that were associated with morning and evening exposure to RF, respectively. These were highly correlated with many different pathways, e.g. multiple transcription factor-regulated pathways, that have been shown to be involved in the modulation of testis development, cell cycle progression, and spermatogenesis.

4.3.6 Oxidative stress

Rasouli Mojez et al. [110] exposed male Wistar rats (n=8 per group) to 900 or 1800 MHz fields for 3 h per day during 28 days. A power density of 0.049-0.317 mW/cm^2 (0.49-3.17 W/m^2) was measured around the brain. They found an increase in the number of dead cells in the hippocampus, as well as an increase in malondialdehyde, and a decrease in the activities of catalase, glutathione peroxidase, and superoxide dismutase, indicating increased oxidative stress. In control groups that performed moderate aerobic exercise (2 × 15-30 min on a treadmill at 1215 m/min speed with 5 min of active recovery between sets) these changes were not observed or of a lesser nature, so they conclude that mild exercise seems to protect against the adverse effects of RF exposure. They also provide SAR values, but these were incorrectly calculated using the external electric field.

Yavaş et al. [111] exposed male Sprague Dawley rats (n=7 per group) to 2100 MHz GSM fields for 5 h per day, 7 days per week, during 14 days. The whole-body SAR was 0.3 W/kg. The total thiol level, the native thiol level and the mean disulphide level in serum were not different from that in the controls. Also, the total antioxidant status, total oxidant status and the oxidative stress index were not increased in the exposed group. So, this treatment did not result in increased oxidative stress.

Vilić et al. [112] exposed larvae of the honeybee (n=32 per group) to 900 MHz fields modulated at 1 kHz, for 2 h at an electric field level of 23 V/m. Glutathione S-transferase activity decreased, and catalase activity increased significantly, while superoxide dismutase activity, the level of lipid peroxidation, and DNA damage were not statistically changed.

4.3.7 Ocular system

Özdemir et al. [113] exposed male Wistar rats (n=16 per group) to 2.4 GHz fields for 2 h per day during 6 weeks, at a SAR in the eye of 0.035 W/kg. Visual evoked potentials (VEP) were lower in the exposed compared to the control group. Ultrastructural analysis showed that axonal diameter and myelin thickness of the optic nerve were also lower. Oxidative stress was increased, since the malondialdehyde level was higher and superoxide dismutase and catalase activities were lower in the exposed group.

4.3.8 Thermophysiology

Mai et al. [114] exposed male Wistar rats (n=4 per group) to 900 MHz RF fields for 23 h per day during 10 days, at a whole-body SAR of 0.35 W/kg. On the 7th day, the ambient temperature was increased from 24 °C to 34 °C by steps of 1 °C every half hour and the tail skin temperature recorded by infrared thermometry. The ambient temperature was thereafter reduced to 24 °C again. On the 10th day, a vasodilator-alpha adrenergic antagonist, prazosin, was injected 30 min before the start of the exposure, the ambient temperature increased to 27 °C and the tail skin temperature measured for 6 h. Rats exposed to RF had lower tail temperature than control rats at ambient temperatures between 27 and 28 °C, suggesting that RF could induce vasoconstriction under mild-warm ambient temperatures. This was suppressed after the injection of a vasodilator. Exposure also led to increased plasma concentrations of noradrenaline (a neurotransmitter responsible for vasoconstriction and thermogenesis) and fatty acids (markers of activated thermogenesis).

4.3.9 Skin

Verma et al. [115] exposed the dorsal skin of male Sprague Dawley rats (n=5 per group) to 10 GHz fields for 3 h per day during 30 days at power densities of 5.23±0.25 and 10.01±0.15 mW/cm^2 (52.3±2.5 and 100.1±1.5 W/m^2). The hair was removed before exposure. With the highest exposure level, the temperature of the skin measured after the last exposure was increased by 1.8 °C. They also observed increased oxidative stress (ROS, 4-HNE, LPO, AOPP), inflammatory responses (NFkB, iNOS/NOS2, COX-2) and metabolic alterations [hexokinase (HK), lactate dehydrogenase (LDH), citrate synthase (CS) and glucose-6-phospahte dehydrogenase (G6PD)]. Changes after the lower exposure level were in the same direction, but it did not show any statistically significant difference compared to sham-exposed controls.

Jin et al [116] exposed male C57/BL6 mice (n=6 per group) to an 1.76 GHz RF LTE signal for 8 h per day, 5 days per week during 4 weeks. The exposure was at a whole-body SAR of 6 W/kg. They observed in the skin a reduced level of γ-H2AX, a marker for DNA damage. They consider this to be an indication for a protective effects of exposure, but an effect of heating with that high exposure level cannot be excluded.

4.3.10 Heart

Yin et al. [117] exposed male Wistar rats (n=5 per group per analysis) to 2.856 and 9.375 GHz fields, either alone or in combination, at power densities of 5 or 10 mW/cm^2 (50 or 100 W/m^2) for 6 min per frequency. At 6 h, 7, 14 and 28 days after exposure the ECG was measured and biochemical and histological analyses were performed. They found that the heart rate increased, the P wave amplitude decreased, and the R wave amplitude increased after exposure. Also, the content of the myocardial enzymes in serum increased and the structure and ultrastructure of cardiac tissue were damaged. In general, more changes were found after exposure to the highest power density, with virtually no differences between the two frequencies. They also found that the expression of the protein Cx43, which plays a role in electrical conduction of the heart, was decreased in exposed myocardial tissue and that its distribution was abnormal.

4.3.11 Hormones and growth factors

Kim et al. [118] exposed pregnant Sprague Dawley rats (n=4-7 per group) to 915 MHz fields at a whole-body SAR of 4 W/kg, for 8 h per day from gestational day 1 to 19. Cortisol in the blood of the dams and in the adrenal gland were significantly increased in the RF-exposed group. Placental cortisol and the level of placental 11β-HSD2 mRNA expression, indicative of the placental barrier, was not changed.

4.3.12 Conclusions

As in previous years, there is again a variety of endpoints with diverging and inconclusive results. This year, most included studies show effects of exposure, a few do not. The exposure parameters, such as frequency, duration and exposure level, again vary considerably between studies. It is therefore difficult to draw general conclusions other than that under certain circumstances some effects from RF EMF exposure are observed in experimental animals. The observations of increased oxidative stress reported in previous SSM reports continue to be found, in contrast to effects on memory and behaviour.

Out of the 52 retrieved studies, 21 had to be excluded from analysis because of various reasons. It is of concern that 12 studies had to be excluded because of a flawed study design (mainly no sham-exposed group) or missing crucial information on dosimetry. Analyses that include all studies regardless of their quality will provide a biased picture. The analyses performed in the WHO review of effects of RF EMF will take the study quality into consideration.

Endpoint	**Reference**	**Species**	**Exposure and duration**	**Results**
Effects on brain	Lin et al. (2021)	ICR mice, male	2 GHz; 1 h/d, 4, 8 wk; 0.5, 1.0, 1.5 W/m^2	Increased apoptosis in hippocampus and cerebral cortex, increased oxidative stress.
	Othman et al. (2021)	Sprague Dawley rats, female	2.45 GHz; 30 min once or daily, 5 d/wk, 10 d; brain SAR 0.069-0.075 W/kg	Decrease in glucocorticoid receptors and heat shock protein in somatosensory cortex, hypothalamus and hippocampus, increase in limbic cortex; no change in glial fibrillary acidic protein.
	He et al. (2021)	BALB/c mice, male	2.45 GHz pulsed (500 pps); 20 min/d, 2, 4, 6 wk; average SAR 6 W/kg	Reduced neurogenesis rate; alterations in microglia, decreased expression of microglial CX3C chemokine receptor 1, but not sirtuin 1.
	Wang et al. (2021)	fruit fly *Drosophila melanogaster*	3.5 GHz; 3 d; 0.1, 1, 10 W/m^2	Expression genes related with heat stress responses increased. Expression genes associated with circadian clock altered, activity neurotransmitters associated with sleep decreased, expression levels associated genes altered, all without clear exposure-related pattern.
Cognition and behaviour	Bayat et al. (2021)	Sprague Dawley rats, male	2.4 GHz WiFi; 2 h/d, 45 d; SAR 0.0060- 0.0346 W/kg	Exposure recovered learning and memory performance, LTP induction, cell loss in

				dementia model, no effect on synaptic transmission.
	Rui et al. (2021)	Sprague Dawley rats, male	5.8 GHz; 2, 4 h; brain SAR 2.33 W/kg	No changes in memory and learning and several factors associated with neuron function and plasticity.
	Delen et al. (2021)	Wistar rats, male	2600 MHz; 30 min/d, 5 d/wk, 30 d; brain SAR_{10g} 0.295 W/kg	Increased oxidative stress, structural deformation, apoptosis in brain tissue. Reduced by melatonin.
	Deniz et al. (2021)	Wistar rats, female	900 MHz; 1 h/d during 21 d (until the end of pregnancy); electric field 19.21 mV/m	No effects on behaviour of pups, decreased number of pyramidal neurons in hippocampus, increased oxidative stress.
	Qubty et al. (2021)	ICR mice, male and female	iPhone 7; 4-5 h/d, 6 wk; electric field 20 V/m	No effects on anxiety, object recognition, decrease spatial memory in females.
	Tan et al. (2021)	Wistar rats, male	1.5 and/or 2.856 GHz; 6 min/frequency; 100 W/m^2	Decrease spatial memory, EEG changes, structural injuries, downregulation enzymes associated with memory processes in brain. Larger changes with exposure to both frequencies.
	Zhu et al (2021)	Wistar rats, male	1.5 and/or 4.3 GHz; 6 min/frequency; 100 W/m^2	Decreased learning, memory decline; increased hippocampal damage. Larger changes with exposure to both frequencies.
	Shai et al. (2021)	Sprague Dawley rats, male	1052-1979 MHz, jammer; 2 h once, 2 h/d, 2 wk; power density 86.18 $\mu W/m^2$	Single exposure: decrease learning time; repeated exposures: decrease learning, memory.
Genotoxicity	Gunes et al. (2021)	fruit fly *Drosophila melanogaster*	900, 1800, 2100 MHz; 2, 4, 6 h, 2 d; electric field 35.2, 41, 41 V/m, respectively	Increase in genotoxicity score (900 MHz: all exposure times; 1800 MHz: 4 h; 2100 MHz: 4 and 6 h).
Cancer	Kolosnjaj-Tabi et al. (2021)	C57BL6 mice, female	1.5 GHz, pulsed; narrow-band: 20 bursts of 5000 pulses, 20 s between bursts, wide-band: 5 bursts of 500 pulses, 115 s between bursts; electric field 35, 200 kV/m, respectively	No effect growth implanted tumours, blood vessel permeability.

Development	Kim et al. (2021)	ICR mice, pups	1800 MHz; 5 h/d, 4 wk; whole-body SAR 4.0 W/kg	Decrease dendritic spines in dentate gyrus, not in cornu ammonis; decrease expression glutamate receptors, brain-derived neurotrophic factor in both; decreased memory.
	Wang et al. (2021)	fruit fly *Drosophila melanogaster*	3.5 GHz; 3 d; 0.1, 1, 10 W/m^2	Increased expression heat shock protein genes, humoral immune system genes, oxidative stress; decreased diversity microbiome; increased rate of development.
	Vargová et al. (2021)	tick *Dermacentor reticulatus*	900 MHz; 30, 60, 90 min; power density 1 mW/m^2	Exposed 60 min: larger dimensions; other groups: smaller.
	Sun et al. (2021)	flatworm *Caenorhabditis elegans*	9.4 GHz, modulated at 10, 20, 50 Hz; 30 min; average SAR 4.33, 8.66, 21.65 W/kg, respectively	No changes in development, movement, egg production, ROS, lifespan.
Fertility	Almášiová et al. (2021)	Wistar rats, female	2.45 GHz; 2 h/d, throughout pregnancy; whole-body SAR 1.82 W/kg	Increased degeneration testicular parenchyma, oxidative stress.
	Andrašková et al. (2021)	Wistar rats, female	2.45 GHz; 2 h/d, throughout pregnancy; whole-body SAR 1.73 W/kg	Increased degeneration testicular parenchyma, decreased proliferation germ cells.
	Dong et al. (2021)	C57BL/6 mice, male	1.5 GHz; 2x15 min; whole-body SAR 3, 6, 12 W/kg	No pathological, ultrastructural changes in testicles, spermatozoa; no change serum testosterone level; increased motion spermatozoa 6 h after exposure, decreased at 1 day, without dependence on exposure level.
	Hasan et al. (2021)	Swiss albino mice, male	2400 MHz; 40, 60 min/d, 60 d; SAR 0.087 W/kg	Decrease body weight, erythrocytes, increase leukocytes, hemoglobin, serum creatinine, more in longer exposed group.
	Qin et al. (2021)	C57BL/6 J mice, male	1800 MHz; 2 h/d, morning or evening, 21 d; whole-body SAR 0.5 W/kg	Decrease testis weight, sperm production, testosterone secretion.
Oxidative stress	Rasouli Mojez et al. (2021)	Wistar rats, male	900, 1800 MHz; 3 h/d, 28 d; power density 0.49-3.17 W/m^2	Increase oxidative stress; protection from moderate aerobic exercise.
	Yavaş et al. (2021)	Sprague Dawley rats, male	2100 MHz GSM; 5 h/d, 7 d/wk, 14 d; whole-body SAR 0.3 W/kg	No changes oxidative stress.

	Vilić et al. (2021)	Honey bee	900 MHz modulated at 1 kHz; 2 h; electric field 23 V/m	Changes some oxidative stress parameters, no DNA damage.
Ocular system	Özdemir et al. (2021)	Wistar rats, male	2.4 GHz; 2 h/d, 6 wk; eye SAR 0.035 W/kg	Decrease visual evoked potentials, increase oxidative stress.
Thermophysiology	Mai et al. (2021)	Wistar rats, male	900 MHz; 23 h/d, 10 d; whole-body SAR 0.35 W/kg	Induced vasoconstriction.
Skin	Verma et al. (2021)	Sprague Dawley rats, male	10 GHz; 3 h/d, 30 d; 52.3±2.5, 100.1±1.5 W/m^2	Increase oxidative stress, inflammation; metabolic alterations.
	Jin et al (2021)	C57/BL6 mice, male	1.76 GHz LTE, 8 h/d, 5 d/wk, 4 wk; whole-body SAR 6 W/kg	Reduced level of γ-H2AX (could be heating effect).
Heart	Yin et al. (2021)	Wistar rats, male	2.856 and/or 9.375 GHz; 6 min/frequency; 50, 100 W/m^2	Increase heart rate, changes EEG, serum myocardial enzymes, damage cardiac tissue. Decrease protein Cx43, (electrical conduction of the heart).
Hormones and growth factors	Kim et al. (2021 BioEM)	Sprague Dawley rats, female	915 MHz; 8 h/d, gestational day 1-19; whole-body SAR 4 W/kg	Increase cortisol in blood, adrenals glands, not in placenta. No change placental barrier.

4.4 Cell Studies

11 studies were published in 2021 and included in this report. They mainly deal with brain development, cell proliferation, DNA damage, apoptosis and oxidative stress.

4.4.1 Brain development and neurite outgrowth

Chen and co-workers (Chen et al, 2021a) [119] evaluated the effect of RF exposure on early stages of brain development. To this purpose they exposed mice embryonic neuronal stem cells (NSCs) differentiated by treatment with poly-L-lysine to 1800 MHz in a GSM Talk-signal mode, 4 W/kg SAR, for 48 h (5 min on/10 min off cycles). The temperature in the exposure chambers was monitored and maintained at 37 ± 0.5 °C. The results of three independent experiments indicated that, by applying RNA sequencing (RNA-seq) techniques, RF exposure induced transcriptomic changes that resulted in an inhibition of neurite outgrowth (sham controls vs. RF exposed cultures: $p<0.01$). Since ephrin type-A receptor 5 (EPHA5) is required for neurite outgrowth during neuron development, the authors used an EPHA5 recombinant protein to treat the cells during RF exposure to antagonize the inhibitory effects of RF on neurite outgrowth. Such treatment reversed the neurite outgrowth in exposed cells, suggesting a key role of EPHA5 in mediating the effects of RF-EMF on neurite outgrowth.

Li and co-workers (Li et al., 2021) [120] investigated the effect of RF exposure at 1800 MHz GSM, 4 W/kg SAR, on neurite outgrowth and the associated role of the Rap1signaling pathway in primary mouse neurons and on a mouse neuroblastoma (Neuro2a) cell line. The experiments were carried out double-blind and temperature was monitored through the exposure. The results of at least three independent experiments indicated that cell viability was not affected by 24, 48 and 72 h exposure (5 min on/10 min off cycles) in both cell types. When cell cultures were exposed for 48 h, a significant decrease in the total neurite length and in the number of primary and secondary branches was detected compared to sham-controls ($p<0.01$). In Neuro2a cells the total neurite length was also decreased ($p<0.01$) but no significant difference in the average number of neurites was detected. Rap1 protein is considered to affect the growth and differentiation of neuronal cells and switch by cycling between an inactive GDB-binding and an active GTP-binding form. The authors investigated if Rap1 activity and the related signal pathways are involved in the RF-induced disturbance of neurite outgrowth. They found no alteration of Rap1 gene and protein expression and a decrease in the active form (Rap1 GTP) in both cell types (for primary neurons: $p<0.05$; Neuro2a cell line: $p<0.01$).

4.4.2 Cell proliferation, cell cycle, DNA damage, apoptosis and gene and protein expression

The effect of long-term exposure to 1800 MHz was investigated by Ding et al (Ding et al., 2021) [121] on Balb/c-3T3 cells (a mouse embryo cell line) to evaluate if the exposure was associated with carcinogenic risk. Cell cultures were exposed/sham exposed four hours per day for 80 days and then cell proliferation, cell cycle, clone formation and DNA replication was evaluated.

RF was given at an average SAR of 8.0 W/kg and and by means of an appropriate heating system temperature was kept at 37°C. The results of at least three independent experiments indicated that RF exposure enhanced cell proliferation but no significant change in cell cycle was detected. Clone formation also remained unaffected by RF exposure, as assessed by plate and soft agar clone formation. A significant increase was also found in migration activity and, by applying the mRNA microarray analysis, a total of 3905 genes resulted differentially expressed compared to sham controls. The significantly expressed genes (fold change≥5) were the ones involved in cell cycle, cell division, and DNA replication. The authors concluded that, although other *in vivo* and *in vitro* studies must be carried out before obtaining firm conclusions, their results suggest a role of long-term exposure to RF in malignant transformation of mouse embryo cells.

In this paper the authors refer to significant or not significant differences between sham and RF exposed samples, but p values are not reported.

Kim et al (2021a) [122] exposed human neuroblastoma (SH-SY5Y) cell lines to 1760 MHz, LTE, 4W/kg SAR, given for 4 h per day for 4 days, to evaluate cell proliferation, cell cycle progression, DNA damage, apoptosis and senescence. Temperature inside the exposure/sham exposure chambers was maintained constant at 37 °C by circulating water. The results of three independent experiments showed a significant decrease in the cell number of exposed cultures ($p<0.05$), although cell morphology was not affected. Cell proliferation, evaluated by measuring the reduction of tetrazolium salt to formazan, was measured after five days from the exposure and also resulted decreased compared to sham exposed cultures. These findings were confirmed by the cell cycle analysis, carried out with flow cytometric techniques: cells in G0/G1 phase were significantly higher in exposed cultures than in sham exposed ones. Moreover, the expression of three cell cycle regulation proteins (p53, p21 and p27) also resulted modified by the exposure.

Cellular senescence was induced by RF exposure, as assessed by measuring the levels of Akt (protein kinase-B) and mTOR (a mammalian target of rapamycin) phosphorylation. The induction of DNA damage and apoptosis was also measured, and no effects were detected, as assessed by measuring protein expression of Bcl2 and Bax (markers of apoptosis) and phosphorylation of histone H2.X (marker of DNA double strand breaks).

Jin et al (2021) [116] employed several mammalian cell lines to evaluate the effect of 24 h exposure to 1762 MHz, LTE, 8W/kg SAR. Temperature inside the exposure chamber was maintained constant at 37 °C by circulating water. Murine melanoma (B16) and the human keratinocyte (HaCaT) cell lines were used to test the induction of cell viability, apoptosis, necrosis and DNA damage. The results of three independent experiments indicated that cell viability (evaluated by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide - MTT) assay) resulted in a slightly reduction of B16 cells (sham controls vs. exposed cultures, $p<0.05$) but not in HaCaT cells. Such a reduction was also detected in both cell types by Western blot analysis of Ki76, a marker of cell proliferation. Apoptosis and necrosis were not induced in both cell lines. Ionizing radiation was used as positive control for the induction of necrosis and worked properly. No increase in DNA damage, evaluated as induction of double-strand breaks by the comet assay, was found in both cell types. In addition, the authors also evaluated the effect of 3 or 24 h RF exposure, given alone or after a treatment with 10 Gy X-ray (HaCaT and B16 cells) and bleomycin (BL; human melanoma MNT-1 cells). The results obtained with the comet assay indicated that the damage induced by the genotoxic agents was reduced when RF was given for 3 h or 24 h after the genotoxic treatments ($p<0.05$).

To confirm the results, the level of Ɣ-H2AX, a DNA damage marker, was determined and western blot analysis and revealed a significant increase in HaCaT and B16 cells by 10 Gy IR irradiation, as expected. In contrast, Ɣ-H2AX level resulted significantly attenuated by 48 h of RF exposure in

B16 cells ($p<0.05$), a similar trend was observed in HaCaT cells, although not statistically significant. Similar results were obtained in the skin tissue of exposed mice. HaCaT and B16 cells were also employed to investigate the expression of nine DNA repair genes following 24 h RF exposure by means of the quantitative real-time polymerase chain reaction (qRT-PCR). In both cell lines, the p53 gene level was upregulated, while the gadd45 gene level was downregulated ($p<0.05$) , and this could be in accordance with the protective effect detected after the genotoxic treatment. The expression of the other genes was different between HaCaT and B16 cells, maybe due to different cell origin (human and murine).
In this paper a clear description of the sham exposure is reported for *in vivo* but not for *in vitro* experiments.

A bystander effect (BE) is defined as the propagation of the effects from cells directly exposed (targeted cells) to non-exposed cells (bystander cells). This phenomenon has been reported in different *in vivo* and *in vitro* models and is triggered by several agents. Zeni et al. investigated whether RF exposure is capable to trigger a bystander phenomenon in a human neuroblastoma cell model (SH-SY5Y), and whether a correlation exists between adaptive response (AR) and BE (Zeni et al., 2021).

Cell cultures were exposed to 1950 MHz, UMTS signal, 0.3 W/kg SAR, for 20 hours and the induction of DNA damage was evaluated by applying the alkaline comet assay on samples exposed to RF alone and on samples pre-exposed and treated with menadione (MD), a known oxidative DNA damage inducer. DNA damage was measured both in directly RF-exposed cells and in cells treated with culture medium deriving from RF-exposed cells. Monitoring of temperature inside cell cultures was carried out and the detected variations between exposed and sham exposed samples were within the accuracy range of the instrument (37 ± 0.3 °C). The results of four independent experiments indicated absence of DNA damage induced by RF alone. In addition, cells directly exposed to RF were capable of reducing the effect of the subsequent treatment with MD ($p < 0.05$), confirming the induction of AR previously reported (Falone et al., 2018). This effect resulted in propagation to bystander cells with a comparable reduction ($p < 0.05$). Moreover, in six independent experiments the intracellular and extracellular levels of hsp70 were measured by western blot in RF- and sham-exposed cells. A slight but not statistically significant increase in the intracellular fraction and a significant increase ($P < 0.05$) in the extracellular fraction of hsp70 levels were detected in RF-exposed samples compared to sham-exposed controls. Such an increase could contribute to the protective effect induced by RF against the MD-induced DNA damage in terms of DNA-repair mechanisms.

In one paper, the effect of higher frequencies was investigated: Shang et al (2021) [123] evaluated the effects of exposure to 100 GHz in primary neurons isolated from the hippocampi of neonatal Sprague-Dawley rats. Cell cultures were exposed at an average power density of 33 mW/cm^2 for 20 min and gene expression was investigated. The temperature in exposed and sham-exposed samples was monitored with an infrared thermometer and no changes were recorded. The analysis of three experiments revealed that 165 genes were differentially expressed between exposed and sham exposed groups, among which 111 genes were upregulated and 54 genes were downregulated by the exposure. According to the results on transcripts obtained by the RNA-seqencing technique, six genes were selected and verified by the qRT-PCR method. Two of them resulted up-regulated and the remaining four were down-regulated. In addition, the expression of several heath shock proteins and apoptosis-related genes was also evaluated and resulted unaffected, suggesting that the altered gene expression is not due to thermal increase or to induction of apoptosis. The authors concluded that, based on functions regulated by the altered genes, the exposure could affect cell mitosis, phospholipid distribution, biomacromolecule interaction processes, synapse development and neural signalling, although they state that further studies are mandatory to better understand the phenomenon.

Poque et al (2021) [124] have used the Bioluminescence Resonance Energy Transfer (BRET) technique, which makes it possible to visualize, in real time, on living cells, the activations of proteins or interactions between proteins. They monitored the Heat Shock Factor type 1 protein (HSF1) which is considered to be both a temperature sensor and the master regulator of the heat shock stress response in eukaryotes. As they expected, using HEK 293 transfected cells expressing their HSF1 BRET probe, they observed a response of HSF1 with RF-associated temperature elevation (acute exposure 30 min, 1800 MHz, CW, 24 W/kg, 3.5 °C increased) or using a known activator of HSF1 (MG-132). When the temperature was regulated (36°C), no activation of HSF1 by signals at 1800 MHz (CW and GSM) and Wi-Fi, at SARs of 1.5 and 6 W/kg was observed. The basal activity of HSF1 was, according to the authors, marginally decreased by a 24-h exposure to CW signals (1.5 and 6 W/kg) and GSM signal (1.5 W/kg). Altogether, their results indicated that RF exposure at an environmental level or in isothermal condition did not impact HSF1 activation capability.

4.4.3 Oxidative stress

Mitochondria contain specific HSP and protease, which help to fold, unfold, or degrade other proteins. When a large number of unfolded or misfolded proteins are accumulated, the reverse signaling pathway from mitochondria to nucleus is activated to increase the expression of nuclear genes encoding mitochondrial proteins, which include chaperones HSP10 and HSP60 and proteases ClpP and ClpX. This process is called mitochondrial unfolded protein response (UPRmt). Xie and co-workers exposed primary mouse bone marrow stromal cells (BMSC) to investigate the induction of UPRmt induced by 900 MHz CW exposure/sham-exposure given four hours/day for five days at power density of 120 $\mu W/cm^2$, corresponding to an average SAR of $2.5x10^{-4}$ W/kg (Xie et al., 2021) [125]. Cells were collected and processed 30 minutes, 4 and 24 h post exposure. The experiments were repeated three times. Since URP activation is strictly related to Jun N-terminal kinase 2 (JNK2) activation, cultures transfected with small interfering RNA (siRNA) for JNK2 silencing were included in the experimental design. In addition, cultures exposed to X-rays were set up as positive controls. When ROS formation was evaluated (flow cytometry) a significant increase was recorded in RF-exposed cultures ($p<0.05$) at 30 min and 4 h post exposure and reversed to sham-control values at 24 h. In X-rays treated cultures, ROS formation was much higher than in RF-exposed ones. The expression levels of HSP10, HSP60, and ClpP proteases increased significantly 30 min and 4 h post exposure ($p < 0.05$) and returned to nearly those of sham-exposed cells at 24 h, indicating that RF could induce UPRmt in BMSCs. In cultures transfected with siRNA for JNK2, the expression levels were significantly decreased compared to the RF group ($P <0.05$), indicating that RF activates UPRmt through the JNK2 signalling pathway.

Kim and co-workers evaluate the effect of RF on human keratinocytes by exposing HaCaT cell lines for 2 h per day for 4 days in the same conditions reported in Kim et al., 2021a to evaluate the induction of reactive oxygen species (ROS) and cellular senescence (Kim et al., 2021b) [126]. Also, in this study temperature inside the exposure/sham exposure chambers was maintained constant at 37 °C by circulating water. From three to six independent experiments were carried out and the results obtained showed no significant differences between sham exposed and RF exposed cultures in terms of cell morphology, cell number and viability. Apoptosis also resulted unaffected by the exposure, as assessed by measuring protein expression of Bcl2 and Bax. At variance, the exposure induced a significant increase in ROS formation ($p<0.001$), as assessed by flow cytometry. ROS production was also induced after exposure to ultraviolet A (UVA), used as positive control. Cellular senescence was evaluated by measuring several skin senescence-related factors, such as the protein expression and activity of matrix metalloproteinases (MMP) and forkhead box O3 (FoxO3) phosphorylation levels. The exposure significantly upregulated the expression of MMP1($p<0.05$), MMP7($p<0.01$), MMP3, MMP2 and MMP9 ($p<0.001$) and increased the FoxO3 phosphorylation levels ($p<0.05$). Also, in this case exposure to UVA was used as positive control and worked properly. The authors concluded that such results suggest that RF exposure would contribute to skin-aging processes.

McNamee et al [127] also evaluated if RF exposure is capable of inducing oxidative stress and altering the activation of certain signal transduction (ST) pathways (McNamee et al, 2021). A human-derived A172 glioblastoma cell line was exposed to 1800 MHz, continuous-wave (CW) or GSM-modulated, for 5, 30 or 240 min at a SAR level of 0 (sham) or 2.0 W/kg. Temperature in the RF-EMF and sham exposed samples differed by less than 0.1 °C. The expression levels of phosphorylated and total-signal transduction proteins (CREB, JNK, NF-jB, ERK1/2, Akt, p70S6K, STAT3 and STAT5) and antioxidant proteins (SOD1, SOD2, CAT, TRX1, PRX2) were assessed in four independent experiments, carried out blinded. All endpoints were assessed in presence and in absence of serum, as it has been reported that ST protein and phosphoprotein responses may be more easily detectable under serum starved culture conditions. The expression levels of antioxidant proteins resulted unaffected by the exposure, except for CAT that resulted lowered after 30 min RF-EMF exposure in serum-containing cultures in relation to the time-matched sham control ($p<0.05$); no differences were observed at other time-points or in any of the serum-free cultures. The relative expression level of signal transduction proteins resulted unaffected by the CW exposure, except for JNK that was lower after 30 and 240 min exposure, and STAT3 that was lower in the exposed culture lysates after 240 min of exposure ($p<0.05$). However, similar changes were not observed in serum-free cultures. In cultures exposed to 1800 MHz GSM-modulated no statistically significant differences were observed in serum-containing cultures, but in serum-free cultures STAT5 relative expression was lower after 30 and 240 min exposure ($p<0.05$). Anisomycin was used as positive control and induced large fold changes.

Table 4.4.1: Cell studies on exposure to radiofrequency fields

Cell type	Endpoint	Exposure conditions	Results	References
Mice embryonic neuronal stem cells (NSCs) n = 3	Neurite outgrowth (RNA-seq)	1800 MHz, GSM 4 W/kg 48h (5 min on/10 min off cycles)	inhibition of neurite outgrowth, reversed by treatment with EPHA5 recombinant protein.	Chen et al. (2021a)
Primary mouse neurons; mouse neuroblastoma (Neuro2a) cell line n = 3	neurite outgrowth, Rap1 signal pathway	1800 MHz, GSM 4 W/kg 24, 48, 72h (5 min on/10 min off)	No effect on cell viability after 24 to 72 h exposure; decrease in the total neurite length and in the number of primary and secondary branches after 48 h exposure in both cell types; no alteration of Rap1 expression; decrease in Rap1 GTP in both cell types.	Li et al (2021)
Mouse embryo cell line (Balb/c-3T3) n = 3	Cell proliferation, cell cycle, clone formation and DNA replication	1800 MHz 8 W/kg 80 dd (4 h/day)	No effects on cell cycle and clone formation; increased cell proliferation and migration activity. Over-expressed genes involved in cell cycle, cell division and DNA replication.	Ding et al. (2021)
Human neuroblastoma (SH-SY5Y) cells n = 3	Cell proliferation, cell cycle progression, DNA damage, apoptosis, senescence	1760 MHz, LTE 4W/kg 4 h/day for 4 days	Decreased cell number and proliferation; cell cycle delay in G0/G1 phase. No effect on DNA damage and apoptosis.	Kim et al. (2021a)

Murine melanoma (B16) cell line; Human keratinocyte (HaCaT) cell line; Human melanoma (MNT-1) cells n = 3	Cell viability, apoptosis, necrosis and DNA damage	1762 MHz, LTE 8W/kg 3, 24, 48 h combined treatments with X-ray or BL	Reduced viability. No effect on apoptosis, necrosis and DNA damage. Reduction of DNA damage induced by X-ray (HaCaT and B16 cells) and BL (MNT-1 cells) by 3 and 24 h RF exposure. Upregulation of p53 and downregulation of gadd45 genes in both cell types. [This paper is also described in *in vivo* section]	Jin et al. (2021)
Human neuroblastoma (SH-SY5Y) cells n = 4 to 6	DNA damage (comet assay); HSP70 levels	1950 MHz, UMTS 0.3 W/kg 20 h combined treatments with MD	No effect of RF alone on DNA damage; reduction of MD-induced DNA damage in cells pre-exposed to RF and in bystander cells. Increased extracellular but not intracellular levels of hsp70.	Zeni et al. (2021)
Primary rat neurons n=3	gene expression	100 GHz 33 mW/cm^2 20 min	111 genes upregulated and 54 downregulated, related to mitosis, phospholipid distribution, biomacromolecule interaction processes, synapse development and neural signaling. No differences for gene related to HSPs and apoptosis.	Shang et al. (2021)
Primary mouse bone marrow stromal cells (BMSC) n=3	ROS formation; UPRmt	900 MHz, CW 2.5x10^{-4} W/kg 4 h/day for 5 days	ROS increase, reverted after 24 h exposure; expression of HSP10, HSP60, and ClpPs increased at 30 min and 4 h after exposure and reverted returned to those at 24 h.	Xie et al. (2021)
Human keratinocyte (HaCaT) cell line n = at least 3	Cell growth, viability, apoptosis, ROS and cellular senescence	1760 MHz, LTE 4W/kg 2 h/day for 4 days	No effect on cell growth, viability and apoptosis. Increased ROS formation, expression and activity of MMP and FoxO3 phosphorylation.	Kim et al. (2021b)
Human-derived glioblastoma (A172) cell line n = 4	Oxidative stress in presence and in absence of serum	1800 MHz, CW, GSM 2 W/kg 5, 30 or 240 min	No effects on antioxidant enzymes expression, except for CAT (lowered after 30 min CW exposure in in presence of serum). No effects of CW exposure on ST protein expression except for JNK (lowered after 30 and 240 min) and STAT3 (lowered after 240 min) in presence of serum). No effect of GSM exposure, except for STAT5 (lowered after 30 and 240 min) in serum-free cultures.	McNamee et al. (2021)
Human embryonic kidney 293 cells (HEK 293) n = 8	Heat shock response in real time	1800 MHz (CW, GSM, and Wi-Fi-modulated signals) 1.5 and 6 W/kg 24 h	No effects on HSF1 activation for all exposure conditions. A slight decrease of basal activity by a 24-h exposure to CW signals (1.5 and 6 W/kg) and GSM signal (1.5 W/kg).	Poque et al. (2021)

Abbreviations: AR: adaptive response; BL: bleomycin; CAT: catalase; ClpPs: CW: continuous wave; EPHA5: ephrin type-A receptor 5; FoxO3: forkhead box O3; GSM: Global System for Mobile Communications; HSPs: heath shock proteins; JNK: c-Jun N-terminal kinases MD: menadione; MMP: matrix metalloproteinases; Rap1 GTP: active form of Rap1; RNA-seq: RNA sequencing; ROS: reactive oxygen species; STAT: signal transducer and activator of transcription; UMTS: Universal Mobile Communication System; UPRmt: mitochondrial unfolded protein response.

4.4.4 Summary and conclusions on RF *in vitro* studies

Also this year, papers published 2021 on *in vitro* studies evaluated several biological endpoints and the results are not univocal, with increase, decrease or no difference compared to sham controls. Although in several cases a difference was recorded with respect to sham-exposed samples, it is mainly related to the cell type investigated and is reversible, and therefore its biological relevance is unclear.

Unfortunately, as in previous years, a number of studies had to be excluded from the analysis, mainly due to the lack of dosimetric information or of sham-exposed cultures to be used as control.

Appendix: Studies Excluded from Analysis

Articles were identified in relevant scientific literature data bases such as PubMed as well as in the specialized database EMF Portal. Reference lists of articles were screened for relevant papers. Several studies had to be excluded from further analysis as they did not fulfil quality criteria. In this Appendix, the excluded studies[4] are listed and the reasons for exclusion are indicated. Treatment-related studies are not listed. The list is divided into epidemiological studies, human studies, animal studies and cell studies.

A.1 Epidemiological studies

In a first step, all articles that were not relevant for this report were discarded, i.e.

A) papers that did not study non-ionizing electromagnetic fields (i.e. static, extremely low frequency, intermediate frequency or radiofrequency EMF), or

B) did not study any health outcome (including letters, commentaries etc.), or

C) did not in any way study the association between (radiofrequency) electromagnetic fields and a health outcome (e.g. use of text messages for self-management of diabetes).

D) Studies on using EMF as therapeutic interventions (e.g. diathermy),

E) Case-reports were also excluded.

F) Further, studies that did not include humans were excluded, as well as studies of humans with an experimental design (these studies are included under "human studies").

G) Not a peer-reviewed publication, or published in another language than English,

H) Studies published outside of the time frame of this report (online publication date).

Further, the following exclusion criteria were applied after screening the abstracts/full text:

I) Study base not identified (e.g. self-selection of subjects in cross-sectional or case-control studies, the population intended for inclusion not described)

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[4] The articles are primarily identified through searches in relevant scientific literature data bases. However, the searches will never give a complete list of published articles. Neither will the list of articles that do not fulfil quality criteria be complete.

J) No comparison group or no exposure considered (either no unexposed group or lacking denominator for prevalence/incidence calculation in descriptive or incidence study), with the exception of incidence trend studies from registries applying a systematic data collection.

K) Narrative reviews

L) Duplicate reports, unless new additional analyses are presented (including the first original publication, and information from duplicate reports if new additional results were presented)

M) Addressing exclusively exposure assessment methods which have been proven to be invalid such as self-estimated distance to mobile phone base stations.

N) Studies on self-reported quality of life outcomes/psychological outcomes and media use if they do not explicitly mention EMF

Acharya et al. [128]	B
Adekunle et al. [129]	J
Aerts et al. [130]	B
Ahmad et al. [131]	K
Alkayyali et al. [132]	F
Al-Khlaiwi et al. [133]	H
Al-Khlaiwi et al. [134]	I
Talleb et al. [135]	B
Ayinmode et al. [136]	H
Baaken at al. [137]	C
Baaken et al. [138]	H
Balawender et al. [139]	H
Bedeloğlu et al. [140]	B
Belpoggi et al. [141]	K
Belpomme et al. [142]	K
Besset et al. [143]	B
Bevington et al. [144]	B
Birks et al. [145]	B
Blay et al. [146]	K
Brzozek et al. [147]	B
Cabré-Riera et al. [148]	H
Carlberg et al. [149]	H

Chen et al. [150]	H
Chen et al. [151]	H
Choi et al. [152]	H
Chen et al. [153]	H
Chountala et al. [154]	K
Cinemre et al. [155]	I
Çöl et al. [156]	M
De Giudici et al. [157]	B
de Vocht et al. [158]	B
Deatanyah et al. [159]	B
Deatanyah et al. [160]	B
Di Ciaula A et al. [161]	K
Dilli [162]	K
Emodi-Perlman et al. [163]	N
Filippini et al. [164]	H
Foster et al. [165]	B
Foster et al. [166]	B
Frank [167]	K
Ghemrawi et al. [168]	N
Grimes et al. [169]	B
Hansson Mild et al. [170]	B
Hardell [171]	B
Hardell et al. [172]	B
Hardell et al. [173]	B
Hartwig et al. [174]	B
Himanshi et al. [175]	K
Hirata et al. [176]	B
Hmielowski et al. [177]	B
Hu et al. [178]	K
Huss et al. [179]	B
Ibrayeva et al. [180]	K

Jargin [181]	K
Jargin [182]	B
Kacprzyk et al. [183]	C
Karipidis et al. [184]	K
Keshmiri et al. [185]	B
Kiouvrekis et al. [186]	B
Kiouvrekis et al. [187]	B
Klimek et al. [188]	K
Klune et al. [189]	F
Lagorio et al. [190]	C
Lai et al. [191]	F
Leszczynski et al. [192]	K
Levent et al. [193]	H
Levitt et al. [194]	F
Levitt et al. [195]	F
Levitt et al. [196]	F
Li et al. [197]	B
Li et al. [198]	B
Li et al. [199]	C
López et al. [200]	B
Maleki et al. [201]	M
Martin et al. [202]	H
Meng et al. [203]	N
Miravet-Garret et al. [204]	B
Mohammed et al. [205]	B
Moskowitz et al. [206]	B
Myung et al. [207]	B
Negi et al. [208]	K
Oftedal et al. [209]	B
Okechukwu et al. [210]	K
Onishi et al. [211]	B

Owolabi et al. [212]	J
Owolabi et al. [212]	B
Pall et al. [213]	K
Panjali et al. [214]	I
Pinto et al. [215]	B
Ramirez-Vazquez et al. [216]	B
Ramirez-Vazquez et al. [217]	B
Rashmi et al. [218]	H
Redmayne et al. [219]	K
Ren et al. [220]	C
Revanth et al. [221]	K
Romualdo et al. [222]	K
Röösli et al. [223]	C
Sawyerr et al. [224]	J
Schuermann et al. [225]	K
Selmaoui et al. [226]	B
Shen et al. [227]	A
Shih et al. [228]	H
Sofri et al. [229]	F
Souques et al. [230]	B
Stam et al. [231]	B
Sterling et al. [232]	B
Stjernholm et al. [233]	A
Szemerszky et al. [234]	A
Tatoń et al. [235]	B
Thielens et al. [236]	F
Tuteja et al. [237]	H
Uche et al. [238]	K
van Wel et al. [239]	B
Verbeek et al. [240]	H
Vijayalaxmi et al. [241]	K

Wang et al. [242]	H
Wood et al. [243]	F
Zaroushani et al. [244]	K
Zeleke et al. [245]	B
Zhang et al. [246]	B

A.2 Animal Studies

Excluded Animal studies (SF, ELF, IF)

Reference	Reason for exclusion
Hsu and Weng [247]	Mechanisms of magnetoreception in honeybees
Khalil [248]	No sham exposure(s), no dosimetry, imprecise description of (lab animal) experimental conditions [rat strain, n/control group, housing conditions]
Matsuda et al. [249]	No sham control. Partly treatment-related: Study addressed an electrostatic-based pest control method.
Shabani et al. [250]	No sham exposure. Partly treatment-related: Neuroprotective effect of Vitamin E on rat neural cells exposed to 50 Hz, 3 mT for 4 h/d for 60 d.
Sieron et al. [251]	Deficiencies in description of exposure and dosimetry, e.g., in the simultaneously ELF-MF and RF-exposed group a mobile phone beneath rats' cage was used.

Excluded animal Studies (RF)

Reference	Reason for exclusion
Malik et al. [252]	No dosimetry, intra-group design
Jelodar, G. et al. [253]	No sham
Dai, Z. et al. [254]	Analgesic effect
Klune, J. et al. [189]	Only exposure assessment
Mansourian, M. et al. [255]	Therapy
Uche, U. et al. [238]	Modelling
Lajevardipour, A. et al. [256]	Laser
Unsal, M. et al. [257]	Treatment
Ohtani, S. et al. [58]	Intermediate-frequency
Tripathi, R. et al. [258]	No sham, no dosimetry
Abufadda, M. et al. [259]	Green laser
Abdollahi, M. et al. [260]	No sham, no dosimetry
Akbari, H. A. et al. [261]	No sham, no dosimetry

Sieroń, K. et al. [251]	Mobile phone under cage
Zosangzuali, M. et al. [262]	No sham
Akbari, H. et al. [263]	no sham, no dosimetry, no information on exposure parameters
Khoshbakht, S. et al. [264]	no sham, no dosimetry, no information on exposure parameters
Mahmoud et al. [265]	No dosimetry, cell phone in cage
Moghadasi, N. et al. [266]	No sham control, incomplete dosimetry
Sharma, A. et al. [267]	Incomplete dosimetry: SAR calculated from external E field, power density not measured at location of animals
Shojaee, M. et al. [268]	Therapy and unobtainable

A.3 Human studies

Extremely Low Frequency (ELF) fields

Reference	Reason for exclusion
Tripathy et al. [269]	Exposure condition(s) not properly described, no sham exposure condition, no blinding, no statistical analysis

Radiofrequency fields (RF)

Reference	Reason for exclusion
Hanzelka et al. [270]	Poorly described study design, most probably no sham exposure condition.
von Klitzing [271]	The study does not fulfill basic criteria for a good quality study: poorly described exposure, no information on the number of subjects (it seems to be a series of three case studies) and the age, no sham control, poorly described study design

A.4 Cell studies

ELF

Reference	Reason for exclusion
Cios, Ciepielak [272]	number of independent experiments not reported
Colciago, Audano [273]	No exposure system description

IF

Reference	Reason for exclusion
Kim, H. M., et al. [274]	No dosimetry, not clear if sham control has been performed
Kim, J. S., et al. [275]	No sham-control
Michno, A., et al. [276]	No sham-control
Mumblat, H., et al. [277]	No sham-control
Patel, C. B., et al. [278]	No dosimetry, no sham-control
Wu, H., et al. [279]	No dosimetry, no sham-control

RF

Reference	Reason for exclusion
Bhartiya [280]	No sham control
Chowdhury, A., et al. [281]	No Sham control; cell phone in on mode
Górski R, et al. (2021) [282]	No sham-control; no dosimetry
Hassanzadeh-Taheri M et al. [283]	No sham-control, no dosimetry
Ioniţă, E., et al. [284]	No sham-control

Kim K et al. [285]	No sham-control
Lamkowski, A., et al. [286]	No sham-control (samples positioned outside exposure system)
Lan, J., et al. [287]	No sham-control
Ozgur, E., et al. [288]	No sham-control (insufficient description of sham/control conditions)
Perez, F. P., et al. [289]	No sham-control
Sueiro-Benavides, R. A., et al. [290]	No sham-control

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