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Research

Recent Research on EMF and Health Risk, Eighteenth report from SSM's Scientific Council on Electromagnetic Fields, 2023

2024:12

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Corrigendum

Dr. Daniele Mandrioli was listed as a co-author in a previous version of this report. We acknowledge the contribution of Dr. Daniele Mandrioli as a member of the SSM Scientific Council from May 2023 until November 2023, when he resigned. Dr. Daniele Mandrioli did not endorse the report or agree to be an author of the report.

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 2022 och fram till och med december 2022. Rapporten är den artonde 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 forskning om sköldkörtelcancer endast i begränsad omfattning.

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 tröskelvärdena för uppfattningsförmågan av elektromagnetiska fält är lägre under hybrida exponeringsförhållanden än under endast DC- eller AC-fältexponering.

Relevans

Resultaten av forskningsöversikten i årets rapport ger ingen anledning att ändra några referensnivåer eller rekommendationer inom 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 exponering av radiovågor från mobiltelefoner. 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.

Behov av vidare forskning

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 vetenskaplig kvalitet har listats. I år, liksom förra året, har många studier uteslutits på grund av för 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.

Projektinformation

Kontaktperson SSM: Karl Herlin
Referens: SSM2024-3158 / 4530606

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 2022 up to and including December 2022. The report is the eighteenth 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.

Relevance

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.

Need for further research

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.

Project information

Contact person SSM: Karl Herlin
Reference: SSM2024-3158 / 4530606

Recent Research on EMF and Health Risk

Eighteenth report from SSM's Scientific Council on Electromagnetic Fields, 2023

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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 scientific developments and provide advice to the authority. In a series of annual reviews, the Council consecutively discusses and assesses relevant new data and places 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 eighteen in the series and covers studies published from January 2022 up to and including December 2022.

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

Anke Huss, PhD, epidemiology, University of Utrecht, the Netherlands (chair)

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

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Florence Poulletier de Gannes, PhD, cell biology, Research engineer, French National Centre for Scientific Research, Talence, France

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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

Epidemiology

Again, last years' summary on static field (or MRI) exposure and health effects is essentially unchanged: Occupational exposure from magnetic resonance imaging (MRI) caused acute and transient symptoms, but long-term consequences for health remained unclear. The new studies that were published and summarised in this report 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

The results from last year's study on lower detection thresholds for hybrid AC and DC electric field exposure (see SSM last year [1]) were supported by a recent study, which systematically investigated perception thresholds for various combinations of direct and alternating current combinations. Even very low combinations of AC and DC field strengths (1 kV/m each) were reliably perceived by at least one participant.

Animal studies

In one study in mice, 31.9 ± 4.5 nT hypomagnetic field (HMF, i.e. less magnetic fields than naturally occuring) exposure for 8 weeks significantly reduced exploration time and impaired the spatial and cognitive memory of mice. In another study in mice, 150 mT static magnetic field exposure for 18 weeks enhanced motility and exploratory activity. In zebrafish, exposure of eggs and prelarvae to 0.1 μT HMF for 13 days, increased embryo mortality, the appearance of abnormal phenotypes, and a significant increase in the embryo's heartbeat rate. A number of non-mammalian studies reported some, but inconsistent, effects in cognition and behaviour, cardiovascular toxicity, reproductive and developmental toxicity, and oxidative stress.

Cell studies

Most of *in vitro* studies dealing with the effect of static fields published in the year 2022 were related to biomedical applications. One study has been recognized but not considered, due to very low number of independent experiments. Two studies were included in the report: one dealing with the effect of static magnetic fields on intracellular calcium (Ca^{2+}), the other looking at cell proliferation. The results suggest that static fields have effect on intracellular Ca^{2+} at 1 mT and can influence cell proliferation through T-type calcium ion channel at 140 mT.

Extremely low frequency (ELF) fields

Epidemiology

In the current reporting period, there was one study addressing ELF-MF exposure and childhood leukaemia. Leukaemia risk was somewhat elevated in children living close to high-voltage power lines, in line with previous reports. The study had also a focus on residential proximity to plant nurseries, which can be found more frequently under high-voltage power lines, with presumably higher pesticide exposure levels. But it did not act as a strong confounder.

A large study on occupational exposure ELF-MF and electricals shock in relation to risk of non-Hodgkin's lymphoma, chronic lymphocytic leukaemia and multiple myeloma did not observe increased risks, but exposure misclassification and healthy worker effects may have affected study results.

Human studies

Studies published in the current reporting period revealed, a) that in an ELF-MF exposure condition more time was needed to reach a specific level of precision in the subjective visual vertical perception as compared to an AC stimulation although the differences were small; b) combinations of very low field strengths of AC and DC electric fields (e.g. 1kV/m each) were reliably perceived by at least one participant. Detection thresholds were significantly lower with increased AC EF strengths, which underline the role of AC in the human perception of hybrid EFs.

Animal studies

Rat studies published in 2022 have shown adverse effects, in particular related to behavioural effects, development and oxidative stress. In particular, two different studies are in agreement with the findings of last year report that showed that exposure to extremely low frequency magnetic (ELF-MF) fields at 50-Hz (0,1-3 mT), increases anxiety-like behaviour. Furthermore, in one study, an adverse effect on activity and exploratory behaviour of ELF-MF exposure has been observed but no effect on social behaviour was described. Two honeybee studies showed adverse effects both in terms of larval development and behaviour. These data are also in agreement with the previous Council report.

Cell studies

In vitro studies published in the year 2022 evaluated the effect of ELF fields on proliferation, DNA damages, ROS production and cell differentiation. Several studies were excluded because they focussed on therapeutic applications or due to quality reasons. Due to the diversity of the chosen cell models, of the exposure conditions and of endpoints, the interpretation of results is challenging. In one study, no effect of ELF exposure was described on cell differentiation in a cell model of leukemia.

Intermediate fields

Epidemiology

The previous report stated that given the very scarce scientific literature on exposure to IF-MF and possible health effects, no conclusions could be drawn. No studies were identified in this year's reporting period and therefore and the same conclusion applies this year.

Human studies

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

Animal studies

In one study in rats, exposure to 150 kHz (0.3 V/cm) resulted in slight adverse effects on some fertility parameters and alteration of hormonal balance.

Cell studies

Only one study has been recognized in 2022 on intermediate frequencies but was not considered due to the scanty quality of the experimental procedure adopted. It is reported in the table of excluded studies.

Radiofrequency fields

Epidemiology

In the last year, most research addressed cancer risks in adults from using mobile phones. Two incidence trend studies did not find indications that brain tumour incidence has increased because of mobile phone use. These studies demonstrate that a risk increase in the range of 20% or higher after 15 years can be excluded. A large prospective cohort study of women did not find an association between mobile phone use and brain tumours.

Two recent large studies have reported a few associations between different aspects of mobile phone use and some semen quality parameters, although the majority of analyses did not indicate increased risks. Given the high number of analyses, this may represent chance findings but needs follow-up investigations.

A prospective cohort study from the Netherlands indicates that EHS is not a very stable attribution and often changes over time, even if the overall proportion in the population may remain constant.

An Iranian study found a decrease of blood pressure in relation to mobile phone use in women but not in men. Given the lack of mechanism and lack of supporting data from other studies, this may be a chance finding or due to residual confounding.

Human studies

One systematic literature review concluded that evidence from human experimental studies in children and adolescents was inadequate to draw conclusions on mobile phone-related exposure effects on brain activity including cognition (Bodewein et al. 2022). Another indicated that there was no consistent relationship between outcome measures (sleep EEG, sleep quality, event related potentials, cognition, behaviour and brain metabolism) and parameters of exposure from different generations (2G, 3G, and 4G) of telecommunication technology (Hinrikus et al. 2022). Based on these results the authors hypothesized that the impact of exposure from 5G in the NR FR1 frequency range (up to 10 GHz) is principally not different from the one of previous generations of mobile communication.

One study pursued to investigate LTE exposure effects on functional connectivity and brain network properties. Results showed that there was no statistically significant difference in terms of static and dynamic functional networks connectivity both in the sham and the LTE exposure condition, which is in line with previous results (SSM last year's report [1]). The result of another study adds to the body of evidence that symptoms including physiological parameters are not causally linked to RF-EMF exposure in subjects with self-reported idiopathic environmental intolerance attributed to EMF (IEI-EMF).

Finally, one study observed a relation between mobile phone exposure and food ingestion and brain energy homeostasis. The study, which has been criticized for several reasons, certainly needs independent replication, necessary to confirm or disprove the presented results and should be performed double-blinded. Sample size for a given power and error probability should be based on the present findings. If more than one real RF exposure condition is involved, order should be considered as a factor in the statistical analysis, and – where applicable – corrections for multiple testing should be considered. Finally, a detailed dosimetric assessment of RF exposure including measurements is necessary.

Animal studies

There is again a variety of endpoints and exposure parameters that were applied in different studies, such as different frequency, exposure duration and exposure level. Most included studies show some effects of exposure, and a few do not. Effects are observed in all endpoints considered: effects on the brain, cognition and behaviour, cancer, development, fertility, oxidative stress, effects on the heart and several other endpoints. Effects are more often observed at relative high, but also at extremely low exposure levels. Therefore, general conclusions on effects of RF-EMF exposure in experimental animals cannot be drawn based only on the studies from year 2022, but it is possible that under certain circumstances effects are induced on different endpoints. It would be better to analyze the data in a different way, per endpoint and including all available data over time. This is currently been done by WHO, and that analysis includes several systematic reviews of major endpoints, such as carcinogenicity and adverse reproductive outcomes (expected in 2024).

Cell studies

Seven studies on the effect of RF exposure on mammalian cell cultures were included in the report. They evaluated the effect of exposure, ranging from 915 MHz to 3 GHz, on a large variety of cellular endpoints, such as proliferation, viability, transformation, DNA methylation, DNA damage, autophagy, apoptosis and oxidative stress. Two studies also considered combined exposure to RF and chemical agents. In some cases, no effects were detected, while in other conditions a variation with respect to sham-controls was recorded, related to the endpoint investigated and the experimental conditions adopted (exposure duration, timing of the biological test applied).

Sammanfattning

Statiska fält

Epidemiologi

Återigen är förra årets sammanfattning om statiska fält (eller MRI) exponering och hälsoeffekter i stort sett oförändrad: Yrkesmässig exponering från magnetisk resonanstomografi (MRI) orsakade akuta och övergående symtom, men långsiktiga konsekvenser för hälsan förblev oklara. De nya studierna som publicerades och sammanfattades i denna rapport utmanar inte den observationen. Med tanke på att exponeringen för statiska fält från MRI är relativt hög, och antalet yrkesmässigt exponerade personer ökar, behövs mer systematisk och omfattande forskning om detta ämne.

Människostudier

Resultaten från förra årets studie om lägre detektionströsklar för hybrid AC- och DC-elektrisk fältexponering (se SSM förra året [1]) stöddes av en nyligen genomförd studie, som systematiskt undersökte perceptionströsklar för olika kombinationer av lik- och växelströmkombinationer. Även mycket låga kombinationer av AC- och DC-fältstyrkor (1 kV/m vardera) uppfattades pålitligt av minst en deltagare.

Djurstudier

I en studie på möss reducerade exponering för 31,9 ± 4,5 nT hypomagnetiskt fält (HMF, dvs. mindre magnetfält än naturligt förekommande) under 8 veckor signifikant utforskningstiden och försämrade mössens spatiala och kognitiva minne. I en annan studie på möss, förbättrade exponering för 150 mT statiskt magnetfält under 18 veckor rörlighet och utforskningsaktivitet. Hos zebrafisk ökade exponering av ägg och förlarver för 0,1 µT HMF under 13 dagar embryo-dödligheten, utseendet av onormala fenotyper och en signifikant ökning av embryots hjärtfrekvens. Ett antal icke-mammala studier rapporterade några, men inkonsekventa, effekter på kognition och beteende, kardiovaskulär toxicitet, reproduktiv och utvecklingstoxicitet och oxidativ stress.

Cellstudier

De flesta in vitro-studier som behandlar effekten av statiska fält publicerade år 2022 var relaterade till biomedicinska tillämpningar. En studie har beaktats men inte inkluderats, på grund av ett mycket lågt antal oberoende experiment. Två studier inkluderades i rapporten: en som handlar om effekten av statiska magnetfält på intracellulärt kalcium (Ca^{2+}), den andra som tittar på cellproliferation. Resultaten tyder på att statiska fält har effekt på intracellulärt Ca^{2+} vid 1 mT och kan påverka cellproliferation genom T-typ kalciumjonkanal vid 140 mT.

Extremt lågfrekventa (ELF) fält

Epidemiologi

Under den nuvarande rapporteringsperioden fanns det en studie som behandlade ELF-MF exponering och barnleukemi. Leukemirisken var något förhöjd hos barn som bodde nära högspänningsledningar, i linje med tidigare rapporter. Studien hade också fokus på bostadsnära plantskolor, som kan hittas oftare under högspänningsledningar, med förmodligen högre bekämpningsmedelsexponeringsnivåer. Men det fungerade inte som en stark förväxlingsfaktor.

En stor studie om yrkesmässig exponering för ELF-MF och elektriska stötar i förhållande till risken för icke-Hodgkins lymfom, kronisk lymfatisk leukemi och multipelt myelom observerade inga ökade risker, men exponeringsfelklassificering och ”friska arbetare”-effekter kan ha påverkat studiens resultat.

Människostudier

Studier publicerade under den nuvarande rapporteringsperioden avslöjade, a) att under en ELF-MF-exponeringssituation krävdes mer tid för att nå en specifik nivå av precision i den subjektiva visuella vertikalperceptionen jämfört med AC-stimulering även om skillnaderna var små; b) kombinationer av mycket låga fältstyrkor av AC- och DC-elektriska fält (t.ex. 1kV/m vardera) uppfattades pålitligt av minst en deltagare. Detektionströsklarna var signifikant lägre med ökad AC EF-styrka, vilket understryker AC:s roll i människans perception av hybrid EF.

Djurstudier

Råttstudier publicerade år 2022 har visat negativa effekter, särskilt relaterade till beteendeeffekter, utveckling och oxidativ stress. Särskilt två olika studier är överens med resultaten från förra årets rapport som visade att exponering för extremt lågfrekventa magnetfält (ELF-MF) vid 50 Hz (0,1-3 mT) ökar ångestliknande beteende. Dessutom har en negativ effekt på aktivitet och utforskningsbeteende observerats, men ingen effekt på socialt beteende beskrevs. Två studier på honungsbin visade negativa effekter både när det gäller larvutveckling och beteende. Dessa data överensstämmer också med tidigare rådsrapport.

Cellstudier

In vitro-studier publicerade år 2022 utvärderade effekten av ELF-fält på proliferation, DNA-skador, ROS-produktion och celldifferentiering. Flera studier exkluderades eftersom de fokuserade på terapeutiska tillämpningar eller på grund av kvalitetsproblem. På grund av mångfalden av valda cellmodeller, exponeringsförhållanden och slutpunkter är tolkningen av resultaten utmanande. I en studie beskrevs ingen effekt av ELF-exponering på celldifferentiering i en cellmodell av leukemi.

Intermediära fält

Epidemiologi

Den tidigare rapporten uppgav att med tanke på den mycket knappa vetenskapliga litteraturen om exponering för IF-MF och möjliga hälsoeffekter kunde inga slutsatser dras. Inga studier identifierades under detta års rapporteringsperiod och därför gäller samma slutsats i år.

Människostudier

Som för de tidigare rapporteringsperioderna fanns det ingen experimentell studie på människa inom det intermediära frekvensområdet.

Djurstudier

I en studie på råttor resulterade exponering för 150 kHz (0,3 V/cm) i lätt negativa effekter på vissa fertilitetsparametrar och förändring av hormonbalansen.

Cellstudier

Endast en studie har beaktats år 2022 om intermediära frekvenser men ansågs inte på grund av den bristfälliga kvaliteten på den experimentella proceduren som användes. Det rapporteras i tabellen över exkluderade studier.

Radiofrekventa fält

Epidemiologi

Under det senaste året har de flesta studier behandlat cancerrisker hos vuxna vid användning av mobiltelefoner. Två incidenstrendstudier fann inga indikationer på att förekomsten av hjärntumörer har ökat på grund av mobiltelefonanvändning. Dessa studier visar att en riskökning i storleksordningen 20 % eller högre efter 15 år kan uteslutas. En stor prospektiv kohortstudie av kvinnor fann inget samband mellan mobiltelefonanvändning och hjärntumörer.

Två nyligen genomförda stora studier har rapporterat några samband mellan olika aspekter av mobiltelefonanvändning och vissa parametrar för sädeskvalitet, även om de flesta analyser inte visade på ökade risker. Med tanke på det höga antalet analyser kan detta representera slumpmässiga fynd men behöver uppföljningsundersökningar.

En prospektiv kohortstudie från Nederländerna indikerar att EHS inte är en mycket stabil tillskrivning och ofta förändras över tid, även om den övergripande andelen i befolkningen kan förbli konstant.

En iransk studie fann en minskning av blodtrycket i relation till mobiltelefonanvändning hos kvinnor men inte hos män. Med tanke på bristen på mekanism och brist på stödjande data från andra studier kan detta vara ett slumpmässigt fynd eller bero på kvarvarande förväxling.

Människostudier

En systematisk litteraturöversikt drog slutsatsen att bevis från mänskliga experimentella studier på barn och ungdomar var otillräckliga för att dra slutsatser om mobiltelefonrelaterade exponeringseffekter på hjärnaktivitet inklusive kognition (Bodewein et al. 2022). En annan indikerade att det inte fanns något konsekvent samband mellan utfallsmått (sömn EEG, sömnkvalitet, händelserelaterade potentialer, kognition, beteende och hjärnmetabolism) och parametrar för exponering från olika generationer (2G, 3G och 4G) av telekommunikationsteknik (Hinrikus et al. 2022). Baserat på dessa resultat hypotetiserade författarna att påverkan av exponering från 5G i NR FR1 frekvensområdet (upp till 10 GHz) i princip inte skiljer sig från den för tidigare generationer av mobilkommunikation.

En studie försökte undersöka LTE-exponeringseffekter på funktionell anslutning och hjärnnätverksegenskaper. Resultaten visade att det inte fanns någon statistiskt signifikant skillnad när det gäller statisk och dynamisk funktionell nätverksanslutning både i sken- och LTE-exponeringsförhållanden, vilket är i linje med tidigare resultat (SSM förra årets rapport). Resultatet av en annan studie lägger till bevis för att symtom inklusive fysiologiska parametrar inte är orsakligt kopplade till RF-EMF-exponering hos personer med självrapporterad idiopatisk miljöintolerans tillskriven EMF (IEI-EMF).

Slutligen observerade en studie en relation mellan mobiltelefonexponering och matintag och hjärnans energihomeostas. Studien, som har kritiserats av flera skäl, behöver definitivt oberoende replikation, nödvändig för att bekräfta eller motbevisa de presenterade resultaten och bör utföras dubbelblindat. Urvalsstorleken för en given styrka och fel sannolikhet bör baseras på de nuvarande resultaten. Om mer än en verklig RF-exponeringsförhållande är inblandad bör ordningen betraktas som en faktor i den statistiska analysen, och - där det är tillämpligt - korrigeringar för multipla tester bör övervägas. Slutligen behövs en detaljerad dosimetrisk bedömning av RF-exponering inklusive mätningar.

Djurstudier

Det finns återigen en variation av slutpunkter och exponeringsparametrar som tillämpades i olika studier, såsom olika frekvenser, exponeringstid och exponeringsnivå. De flesta inkluderade studier visar några effekter av exponering, och några gör det inte. Effekter observerades i alla beaktade slutpunkter: effekter på hjärnan, kognition och beteende, cancer, utveckling, fertilitet, oxidativ stress, effekter på hjärtat och flera andra slutpunkter. Effekter observeras oftare vid relativt höga, men också vid extremt låga exponeringsnivåer. Därför kan inga generella slutsatser om effekter av RF-EMF-exponering i experimentella djur dras endast baserat på studier från år 2022, men det är möjligt att under vissa omständigheter så kan effekter induceras på olika slutpunkter. Det skulle vara bättre att analysera data på ett annat sätt, per slutpunkt och inkludera alla tillgängliga data över tid. Detta görs för närvarande av WHO, och den analysen inkluderar flera systematiska översikter av stora slutpunkter, såsom karcinogenicitet och negativa reproduktiva utfall (förväntat 2024).

Cellstudier

Sju studier om effekten av RF-exponering på däggdjurscellkulturer inkluderades i rapporten. De utvärderade effekten av exponering, som sträckte sig från 915 MHz till 3 GHz, på en stor variation av cellulära slutpunkter, såsom proliferation, livskraft, transformation, DNAmetylering, DNA-skador, autofagi, apoptos och oxidativ stress. Två studier beaktade också kombinerad exponering för RF och kemiska agenter. I vissa fall upptäcktes inga effekter, medan under andra förhållanden observerades en variation jämfört med sken-kontroller, relaterat till den undersökta slutpunkten och de antagna experimentella förhållandena (exponeringstid, tidpunkt för det biologiska testet som tillämpades).

Preamble

In this preamble, we explain the principles and methods 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 of eventually weighing 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 of 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 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 important to evaluate both studies indicating that exposure to electromagnetic fields has an effect and 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 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

[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.

and methods for assessing biological or health endpoints 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 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 to 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 have to be supported by experimental studies to establish a causal link between exposure and health. Where this is relevant and possible, effects on other species are also 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 leukemia. It is widely agreed that epidemiology consistently demonstrates an association between exposure to ELF magnetic fields and an increased occurrence of childhood leukemia. However, there is a lack of support for a causal relation from observations in experimental models, and a plausible biophysical mechanism of action is missing. This has led the International Agency for Research on Cancer (IARC) to the overall evaluation of ELF magnetic fields as "possibly carcinogenic to humans" (Group 2B).

[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.

1.1.1 Symptoms

Rathebe et al. [2] assessed risk perception and symptoms among hospital workers working with MRI scanners in two South African hospitals with either a 1.5 or a 3T scanner. The study included 77 employees of radiology departments (clinicians, radiographers, cleaners, maintenance engineers, nurses) comparing those working with MRI to those using x-ray or CT scanners. The response rate was 91%. Participants were aged 21-61, 57% were women; on Fridays after the last work shift all filled in a questionnaire pertaining to MRI-scanner room exposure and symptoms during the past work week. Because 60% of the exposed group but none of the unexposed group reported symptoms, only 50 exposed persons were included into the final analysis of associations when being exposed (i.e. entering the MRI scanner room). In addition, the authors evaluated exposure categories of being present in the MRI scanner room during image acquisition, head or upper body movements in the scanner bore, as well as a range of possible effect modifiers.
A set of "control" symptoms (e.g., earache) that had been added to the questionnaire were not reported by participants and these items were excluded from the analysis. Presence in the scanner room during image acquisition was associated with the sensation of skin glowing, movement of head or upper body in the scanner bore with nausea and vertigo, instability when standing and sensing a metallic taste. No correlation between reporting of symptoms and perceived risk of scanners was found.
This study is in line with previous reports of workers experiencing symptoms when they access the MRI scanning room, especially during image acquisition or when performing head/upper body movements in the scanner bore. Weaknesses relate to the small study sample, and the self-reporting of the exposure categories. The authors highlight that personal measurements could be helpful in future assessments.

Glans et al. [3] performed a survey in 2015/2016 in Swedish hospitals that had an MRI unit. Questionnaires were snowballed via 92 hospitals to any personnel working to any degree with MRI or CT scanners. At the time, the number of eligible persons was estimated to be approximately 620 persons working with MRI and 1300 with CT scanners (some with both). Participants (primarily radiographers) were asked how often they experienced vertigo/dizziness, nausea, metallic taste, illusion of movement, ringing sensation or sound (tinnitus), headache, unusual drowsiness or tiredness, forgetfulness, difficulties concentrating, and difficulties sleeping. Exposure categorisation was based on working with MRI only, CT only, or mixed. Further analyses assessed working with <=1.5T scanners vs >1.5T scanners. 546 persons filled in the questionnaire and the response rate was estimated by the authors to be around 60% among personnel working with MRIs. Respondents were excluded if they worked less than 20h/week, which left 342 participants for the final analysis. Of these, 121 worked primarily with MRI, 75 primarily with CT, and 146 with both technologies. Symptom prevalence did not differ significantly between radiographers working with MRI or CTs or the mixed group but working with stronger MRI scanners (≥ 3T) doubled the odds of experiencing symptoms associated with static magnetic field exposure more than once per week (OR 2.03, 95%CI

1.05-3.93). The authors also report that among radiographers' with work stress, more symptoms were experienced when they were exposed to MRIs (effect modifier). About half of the radiographers reported preventive measures, most of them to walk or move slowly in the scanner room or when being close to the gantry.
Strength of the study is the relatively large sample size as compared to previous reports. Limitations pertain to the self-reports of exposure that is limited to hours of working with MRI and the strength of the MRI scanner. The method used to distribute the questionnaires may have resulted in selection bias as exposed workers with symptoms may have been more prone to respond. Overall, the study is in line with previous reports on radiographers experiencing symptoms when exposed to stronger MRI scanners in the scanner room or close to the bore, that exposed workers develop mitigation strategies, and that stress is another underlying factor in developing symptoms.

1.1.2. Other outcomes

Anand et al. [4] investigated the association of solar and geomagnetic activity (SGA) on pulmonary function directly and as an effect modifier in relation to air pollution in the form of particulate matter < 2.5µm ($PM_{2.5}$) and black carbon. The study population was 726 healthy, older male members of the Normative Ageing Study, who every few years between 2000 and 2017 had a clinic visit in Boston including a standardized questionnaire and measurement of forced expiratory volume (FEV) and forced vital capacity (FVC). SGA in the form of daily solar spot number (SSN), interplanetary magnetic field (IMF) and the Kp Index which describes level of disturbance of earths geomagnetic field and air pollution levels from a central Boston location were averaged over periods of 0 to 28 days before clinic visit. The mean daily levels for each were: IMF=5.8 nT, SSN=83.8 and Kp index=18.6. 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. For effect modification analysis the association of air pollution at the highest and lowest 10% of SGA were calculated. Except for Kp-index and FEV, SGA was associated with reduced FEV and FVC and slightly increased FEC/FVC. Effects tended to increase with increasing averaging periods. A one IQR (interquartile range) higher SSN (averaged over 28 days) was associated with a FVC decrease of 173.7 ml (95%CI: 142.6-204.9), a FEV decrease of 116.1 ml (95%CI: 91.0-142.1) and an FEC/FVC increase of 0.4% (95%CI: -0.1-0.8). Effects of one IQR higher $PM_{2.5}$ or BC was stronger when SGA was high (>90th percentile) than when it was low (<10th percentile). This effect modification was most conspicuous for IMF and BC. The authors concluded: "Increased periods of solar and geomagnetic activity may directly contribute to impaired pulmonary function and may also enhance effects of $PM_{2.5}$ and BC."
The authors do not discuss he clinical relevance of the observed effects and it is not clear if any of these effects are directly due to the magnetic field or due to indirect effects e.g., on atmospheric chemistry. Further, it remains unclear whether these findings are relevant for artificial electromagnetic fields with different characteristics in terms of frequency, intensity and temporal variability.

1.1.3 Conclusions on epidemiological studies

Again, last years' summary on static field (or MRI) exposure and health effects is essentially unchanged: Occupational exposure from magnetic resonance imaging (MRI) caused acute and transient symptoms, but long-term consequences for health remained unclear. The new studies that were published and summarised in this report 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.

Table 1.1: epidemiological studies on static fields and health

Endpoints	Reference	Exposure assessment	Study design, Population	Results
Lung function	Anand et al, 2022	Interplanetary magnetic field, Sunspot number and Planetary K Index data from NASA. Air pollution from a single measurement station.	Cohort: 726 older male members of Normative ageing Study, Boston USA, 2000-2017	Lung function indices changed with solar and geomagnetic activity (SGA), effects of air pollution on lung function possibly stronger in conditions of high SGA.
Symptoms	Glans et al, 2022	Self-reported MRI occupational exposure	Survey, 342 population working with MRI or CT in hospitals,	Higher symptom prevalence among employees working with stronger MRI (≥3T). Stress was an effect modifier (more symptoms when exposed and stressed).
Symptoms	Rathebe et al, 2022	Self-reported MRI occupational exposure	Survey, 77 employees working in radiology departments, aged 21-61, 57% women	Presence in the scanner room during image acquisition and movement of head or upper body in the scanner bore correlated with symptoms like nausea and vertigo. No correlation between reporting of symptoms and perceived risk of scanners.

1.2 Human studies

In 2021 Kursawe et al. [5] published the results of a systematic investigation of perception thresholds of electric fields (EF) for alternating currents (AC), direct currents (DC) and hybrid exposure (see also SSM report from last year [1]). Since the results revealed that perception thresholds were lower in the hybrid exposure condition than in the AC and DC exposure alone, Jankowiak et al. [6] further investigated the role of AC for perception in the hybrid exposure condition. The results, which further underline that increased AC EF strengths in combination with various DC field strengths lower detection thresholds, have been discussed in detail in chapter 2.2 (see also table 1.2). This was the only study related to static fields in 2022.

1.2.1 Conclusion human studies

The results from last year's study on lower detection thresholds for hybrid AC and DC electric field exposure (see SSM last year [1]) were supported by a recent study, which systematically investigated perception thresholds for various combinations of direct and alternating current combinations. Even very low combinations of AC and DC were reliably perceived by at least one participant.

Table 1.2: Human studies on exposure to static (and low frequency electric fields - EF)

Endpoints	Reference	Exposure condition	Sample	Results
Perception of hybrid AC and DC exposure	Jankowiak et al. (2022)	Condition 1: AC EF strength 1 kV/m in combination with DC EF strength 1, 2, 4, 8, 16 kV/m. In conditions 2 to 4 an AC EF of 2, 3, and 4 kV/m were combined with the DC EF fields.	51 (20 men and 31 women, age range: 23 to 77 years, mean ± SD: 49.14 ± 17.52 years)	Combinations of 1kV of AC and DC electric fields were reliably perceived by at least one participant. Detection thresholds were significantly lower with increased AC EF strengths, which underline the role of AC in the human perception of hybrid EFs.

1.3 Animal studies

Last years' report stated that most or all studies reported some, although inconsistent, effects. It was also reported that most studies were not hypothesis-driven.

For the reporting year 2022, three experimental studies in mice were identified. In addition, eight papers described studies involving a variety of different non-mammalian species, including fish, molluscs, crustaceans, amphibians and nematodes. The studies explored a wide range of outcomes including: cognition and behaviour, cardiovascular toxicity, reproductive and developmental toxicity, oxidative stress.

1.3.1 Cognition and behaviour

Tian et al. [7] exposed 7-week-old male C57BL/6J mice (n=10 per group) to a geomagnetic field (GMF) and to a 31.9 ± 4.5 nT hypomagnetic field (HMF), produced using a double-wrapped coil system. For GMF control, the magnetic field intensity was 55,548.5 ± 12.8 nT (mean ± SEM). The exposure lasted 8 weeks. Behavioral analyses were carried out in GMF- and HMF- exposed mice at 0 and 8 weeks after magnetic field exposure. Open field test and object location task (OLT) and the novel object recognition task (NORT) test were used. The hippocampus of 4 animals per group was collected and subjected to analysis.

There were no behavioral differences between the GMF and HMF groups before the magnetic field exposure (0 w), but after 8 weeks, the exploration time in HMF mice was significantly reduced compared to the GMF mice showing anxiety. OLT or NOR test data suggested that the HMF exposure impaired the spatial and cognitive memory of mice. The authors also observed that reactive oxygen species (ROS) levels of this area were significantly higher in HMF-exposed mice. PCR array analysis revealed that the elevated ROS levels were due to HMF-regulating genes that maintain the redox balance as Nox4 and Gpx3. Since high levels of ROS may cause hippocampal oxidative stress, HMF exposure could induce cognitive impairment, besides hippocampal neurogenesis impairments.

1.3.2 Oxidative stress

Dong et al. [8] exposed 4-week-old male ICR mice (n=34 per group) to a 56.3kV/m static electric field (SEF) or sham, for 7, 14 and 21 days. They investigated the effects on the mice spleen in terms of oxidative stress indices. Furthermore the cellular ultrastructure of the spleens was observed after the exposure of 21 days.

They observed that SEF exposure of 21 days significantly increased malonic dialdehyde content, superoxide dismutase activity, calcineurin activity, nitric oxide synthase (NOS) activity, and the mRNA expression levels of tumor necrosis factor-α (TNF-α) and nuclear factor-κB (NF-κB) in the spleen. Exposure caused ultrastructural changes of splenic lymphocytes: separation of nucleus and nuclear membrane, disappearance of mitochondrial membrane, and deficiency of mitochondrial cristae. Increased activity of NOS induced oxidative stress of the spleen. The increase of the mRNA expression levels of TNF-α and NF-κB contributed to the occurrence of spleen inflammation.

1.3.3 Other endpoints

Yang et al. [9] exposed eight-week-old female C57BL/6J mice (n=6 per group) to 150 mT static magnetic field using a magnetic plate with polarity (N or S) thus forming the upward (N) or downward (S) magnetic plate, 24 h/day, for 18 weeks. Mice were divided into 3 groups randomly with 6 mice in each group. Authors measured locomotor and exploratory activity by behavioral tests, including the

open field and elevated plus test.
They observed that exposure enhanced the motility and exploratory activity: compared to the sham group, especially downward 150 mT SMF increased the number of entrances to the center area by 67.0% ($p = 0.0082$) and time in the center area by 77.12% ($p = 0.0054$).

1.3.4 Studies in Non-Mammalians

Krylov et al. [10] tested the effect of magnetic field on circadian rhythm of zebrafish. They exposed zebrafish (AB strain) eggs and prelarvae (n=200) to 0.1 µT hypomagnetic field (HMF), or to a geomagnetic field (GMF), using a coil system consisting of three pairs of mutually orthogonal Helmholtz coils. They carried out 2 studies both with 13 days of exposure, from the second to the 116th hour post fertilization. Zebrafish embryos were under 16 h light/8 h dark cycle in a first study and under constant illumination during a second experiment.
They observed that HMF exposure in both lighting modes led to increased embryo mortality, the appearance of abnormal phenotypes, and a significant increase in the embryo's heartbeat rate. The difference between maximal and minimal heartbeat intervals, maximal to minimal heartbeat intervals ratio, and the coefficient of variation of heartbeat rate were increased in the embryos exposed under constant illumination from 96 to 116 h post fertilization. Heartbeat rate changes following a circadian pattern were observed in all studied groups except zebrafish exposed to HMF under constant illumination.

Krylov et al. [11] carried out a second study. They exposed *Daphnya magna* crustaceans (n=22 per group), using Helmholtz coils. The crustaceans were split into four groups of 22 animals in the following conditions: geomagnetic field of 52 µT (control), magnetic field of 5.2 µT, alternating magnetic field of 9.4 µT, 4 Hz combined with the reduced magnetic field of 5.2 µT; alternating magnetic field of 94 µT, 40 Hz combined with the geomagnetic field of 52 µT. Experiments were carried out for 71 days, 24h (except 30 minutes for the evaluation of live females and the number of offspring), until the natural death of all crustaceans.
Exposure to both combinations of magnetic fields led to a long-term delay of the first brood release, an increase in the brood size, a decrease in the number of broods, and the period between broods. A significant reduction of amylolytic activity, proteolytic activity, and sucrose activity was also observed.

Cresci et al. [12] performed two studies to evaluate the effect of submarine electromagnetic fields, produced by cables used for various types of anthropogenic infrastructures on marine life forms. In the first of this study they exposed Atlantic haddock (*Melanogrammus aeglefinus*); two females were used as a source of eggs that were fertilized and placed into one 500 litres tank. They used 92 larvae (n=46 per group) of 32-33 days after the hatch. One group of larvae was exposed to a 50-150 µT MF gradient, generated by a two square Helmholtz coils, for 10 minutes in a raceway tank. This field simulate that one produced by the submarine high voltage direct current cables. The behaviour of animals was evaluated individually: fish length, position along the x-axis, and median and maximum swimming speed and acceleration were tested. A second group of larvae was not exposed.
The authors found that exposure did not affect the spatial distribution of haddock larvae in the raceway. The majority (78%) of larvae were nonexploratory, and exposure reduced their median swimming speed by 60% and decreased their median acceleration by 38%. There was no effect on swimming of the smaller proportion (22%) of exploratory larvae.

Cresci et al. [13] in a second study, similar to previous one, exposed lesser sandeel (*Ammodytes marinus*) larvae (n=28 per group) to a 50-150 µT MF gradient, for 15 minutes, using the same experimental apparatus of the previous work. Also in this case, half of the animals were exposed and

half used as control group and sham exposed. Larvae's behaviour was tested individually to evaluate in particular their swimming kinematics (average and maximum speed, and acceleration).
The authors found that exposure did not affect the spatial distribution of sandeel larvae in the raceway. Exposure did not reduce their swimming speed, acceleration or distance moved. Although the authors found no impact of magnetic field on larvae kinematics, they reported some changes in behaviour with ontogeny, starting from when they reach 2–3 cm total body length. Thus, larger lesser sandeel might respond differently than larvae to changes in magnetic field.

Ren et al. [14] exposed *Xenopus laevis* tadpoles (n=12-18 per group) to geomagnetic field (sham and blank group) or to static magnetic field (SMF) that generated magnetic gradients of 0.1–30 mT and 0.1–80 mT, for 10 and 6 min. They investigated the effects of a static magnetic field on the locomotion of Xenopus tadpoles. They observed that, compared to the sham control treatment, the presence of a magnet inhibited the movement under any test condition (reductions in swimming distance, speed, and counts of path adjustment). They also observed that SMF was able to alter the tadpoles' movement angle adjustment preference pattern in the dark.

Harsanyi et al. [15] wanted to evaluate the effects of subsea power cables on submarine life. They exposed *Homarus gammarus* lobsters (n=6 per group) and *Cancer pagurus* crabs (n=6 per group) to 2.8 mT static (Direct Current, DC) MF (produced by a Helmholt coil) or sham, throughout embryonic development, for the entire duration of egg incubation and larval release. Embryonic and larval parameters, deformities, and vertical swimming speed of freshly hatched stage I lobster and zoea I crab larvae were assessed.
The authors found that static magnetic fields did not alter embryonic development time, larval release time, or vertical swimming speed for either species. Exposure during egg development resulted in significant differences in stage-specific egg volume and resulted in stage I lobster and zoea I crab larvae exhibiting decreased carapace height, total length, and maximum eye diameter. An increased occurrence of larval deformities was observed in addition to reduced swimming test success rate amongst lobster larvae. These traits affect larval mortality, recruitment and dispersal. SMF exposure did not appear to impact embryonic development time, larval release time, or vertical swimming speed for either species, or crab larval deformities and swimming test success.

Yang et al. [16] studied the effects of high static magnetic field on the reproduction system. They exposed *Caenorhabditis elegans*, sperm and offspring, to a 10T static magnetic field (SMF) for 0 h, 3 h, 5 h, and 12 hs. The strains used in this study were DR466 (him-5, male mutant strain of *C. elegans*) and CB4108 (fog-2, a mutant strain of hermaphrodites *C. elegans*). Morphology, size, activation of sperm were studied. Furthermore, brood size assay and lifespan assay were performed.
The authors explored the biological responses of SMFs exposure at an intensity of 10 T on the sperms and their offspring in him-5 male mutants. They observed that exposure to 10 T SMF reduced the diameter of the inactivated sperms and accelerated the activation of sperms in vivo. The non-transferred ratio was increased in exposed male worms, leading to the decrease of the brood size and eventually diminishing the reproductive capacity of him-5 male worms. The lifespan of outcrossed offspring from exposed him-5 male mutants and unexposed fog-2 female mutants was decreased by 10 T SMF in a time dependent manner.

Fei et al. [17] also evaluated effects of submarine power cables on non-mammalians. They exposed *Elysia leucolegnote* (n=10 per group) to a 1.1 T static magnetic field or geomagnetic field for 10 days, to investigate whether magnetic fields could interfere with the physiological functions of offshore molluscs.
The blood glucose, lipid levels, and activities of antioxidant enzymes were significantly increased upon the exposure to high static magnetic field. Activities of enzymes related to digestive performance

and liver functions were instead decreased. To further investigate these findings, the authors used comparative transcriptome analysis. A total of 836 differentially expressed genes were identified, 352 of which were up-regulated and 484 of which were down-regulated after exposure to 1.1T static magnetic field. The up-regulated differential genes were mainly concentrated in lysosomal and apoptotic pathways, and down-regulated differential genes were mainly involved in digestive and immune systems.

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

In mice, 31.9 ± 4.5 nT hypomagnetic field (HMF) exposure for 8 weeks significantly reduced exploration time and impaired the spatial and cognitive memory. In mice, 150 mT static magnetic field exposure for 18 weeks, enhanced motility and exploratory activity. In zebrafish, exposure of eggs and prelarvae to 0.1 μT HMF for 13 days resulted in increased embryo mortality, the appearance of abnormal phenotypes, and a significant increase in the embryo's heartbeat rate. A number of non-mammalian studies reported some, but inconsistent, effects in cognition and behaviour, cardiovascular toxicity, reproductive and developmental toxicity, and oxidative stress. Studies were generally performed on small groups of animals and long-term studies were missing.

Table 1.3: Animal studies on exposure to static fields

Endpoints	Reference	Exposure condition	Species, strain, sex	Results
Rodent studies				
Cognition and behaviour	Tian et al. (2022)	31.9 ± 4.5 nT 8 wks	Mice, C57BL/6J, male	Reactive oxygen species levels significantly higher in HMF-exposed mice. Cognitive impairment and hippocampal neurogenesis impairments. GMF maintain hippocampal function by regulating the ROS levels.
Oxidative stress	Dong et al. (2022)	56.3 kV/m 7, 14 and 21 d	Mice, ICR, male	21days exposure significantly increased malonic dialdehyde content, superoxide dismutase activity, calcineurin activity, nitric oxide synthase activity, and the mRNA expression levels of tumor necrosis factor-α and nuclear factor-κB in the spleen. Ultrastructural changes of splenic lymphocytes. Occurrence of oxidative stress of the spleen and spleen inflammation
Other endpoint	Yang et al. (2022)	150 mT static magnetic field with polarity (N or S) 24 h/d, 18 wks	Mice, C57BL/6J, female	Enhanced motility and exploratory activity Exposure acted upon mental status and gut microbiota by modulating glucose metabolism and increasing the level of beneficial flora and antioxidant capacity. Upward 150 mT improved number of follicles.

Studies in Non-Mammalian				
Effects on heart	Krylov et al. (2022a)	0.1 μT from the 2nd to the 116th hour post fertilization	Fish (*Zebrafish (AB strain*))	Increased embryo mortality. Increase appearance of abnormal phenotypes and significant increase in heartbeat rate in both lighting modes. Increase in the difference between maximal and minimal heartbeat intervals, maximal to minimal heartbeat intervals ratio, and the coefficient of variation of heartbeat rate after exposure under constant illumination after 96h post fertilization. Heartbeat rate changes following a circadian pattern observed in all groups except exposed to HMF under constant illumination
Cognition and behaviour	Cresci et al. [18]	50 to 150μT MF gradient 10 min	Fish (*Melanogrammus aeglefinus*)	Normal spatial distribution of larvae, reduced median swimming speed by 60% and median acceleration by 38%. 78% of larvae non exploratory.
	Cresci et al. [18]	50 to 150μT MF gradient 15 min	Fish (*Ammodytes marinus*)	Normal spatial distribution of sandeel larvae in the raceway, not reduced swimming speed, acceleration or distance moved.
	Ren et al. (2022)	0.1–30 mT and 0.1–80 mT gradient along the central-to-peripheral direction. 10 and 6 min	Tadpoles (*Xenopus*)	Reductions in swimming distance, speed, and counts of path adjustment in exposed tadpoles. Altered movement angle adjustment preference pattern in the dark after exposure.
Development	Harsanyi et al. (2022)	2.8 mT entire duration of egg incubation and larval release	Lobster (*Homarus gammarus*); Crab (*Cancer pagurus*)	Significant differences in stage-specific egg volume. In stage I lobster and zoea I crab larvae decreased carapace height, total length, and maximum eye diameter. No changes in embryonic development time, larval release time, or vertical swimming speed for either species. Increased occurrence of larval deformities and reduced swimming test success rate in lobster larvae.
Fertility	Yang et al. (2022)	10 T 0, 3, 5 and 12 h	Nematode (*Caenorhabditis elegans him-5and fog-2 mutants*)	Reduced diameter of the unactivated sperms and accelerated activation of sperms in vivo. Decrease of the brood size. Shorter lifespan of outcrossed offspring.
Oxidative stress	Fei et al. (2022)	1.1 T	Mollusc	Increased oxidative stress and blood glucose and lipid levels,

		10 days	(*Elysia leucolegnote*)	and decreased activities of enzymes related to digestive performance and liver functions.
Other endpoint	Krylov et al. (2022b)	5.2 μT static magnetic field and 4Hz 9.4 μT alternating magnetic field; 52 μT static magnetic field and 40Hz 94 μT alternating magnetic field 71 days	Crustacean (*Daphnya Magna*)	Long-term delay of the first brood release, increase in the brood size, decrease in the number of broods, and period between broods after both combinations of magnetic field. Significant reduction of amylolytic activity, proteolytic activity, and sucrase activity.

Abbreviations: d: day(s); GMF: geomagnetic field; h: hours; HMF: hypomagnetic field; min: minute; ROS: reactive oxygen species; SMF: static magnetic field; wk: week(s)

1.4 Cell studies

Last year, only one study was taken into account suggesting that static fields would be able to induce variations in the biological endpoints considered (proliferation and ROS production) depending on the intensities (between 0.5 and 600 µT).

In the current report, two papers were taken into account. Effects of both RF (see RF section) and SMF were tested on intracellular calcium (Ca^{2+}), considered as a central modulator in various signalling pathways, in human embryonic kidney cell line HEK 293 (n=3 experiments) (Bertagna et al. [19]). Cells placed in an incubation room at 38°C were exposed with two Helmholtz coils at 1 mT for 30 min. Intracellular Ca^{2+} was monitored thanks a Fluo-4AM Ca^{2+} dye. The sham plate was subjected to the same conditions of the exposure groups, but the SMF generator was off. A significant increase in fluorescence reflecting an increase in Ca^{2+} concentration was observed after 30 min of exposure (compared to sham) but not after 15 min. The temperature was monitored during the whole experiments.

Human mesenchymal stem cells (MSCs) exposed to SMF (72 h at 140 mT) (Wu et al. [20]) have shown increase in proliferation by 23% (if compared to those without an external applied static magnetic field) (n=3) and activation in the expression of cell-growth related genes such as FOS (Fos Proto-Oncogene) and EGR1 (Early Growth Response 1) via activation of the MAKP signalling pathway. The involvement of the latter was suggested by the expression of signal-transduction proteins p-ERK1/2 and p-JNK that oscillated periodically with SMF exposure time. The authors found that the inhibition of the T-type calcium ion channels with a T-type Ca^{2+} channel blocker (CCB), mibefradil dihydrochloride negates the biological effects of SMFs on MSCs. Together, this paper revealed that the SMFs regulate T-type calcium ion channels and mediate MSC proliferation via the MAPK signalling pathways.

1.4.1 Conclusions on static field cell studies

The first *in vitro* paper on SMF effect on intracellular Ca^{2+} in cells complement the literature on this topic. The second paper links SMF exposure increase in cell proliferation to T-type calcium ion channel.

Table 1.4: Cell studies on exposure to static magnetic fields

Cell type	Endpoint	Exposure conditions	Results	References
Human embryonic kidney cell line HEK 293 n=3	Calcium	1 mT 30 min	Increase in calcium concentration after 30 min of exposure	Bertagna et al. (2022)
Human mesenchymal stem cells (MSCs) n=3	Proliferation	140 mT 72h	Increase of cell proliferation after 72 h of exposure	Wu et al. (2022)

2. Extremely low frequency (ELF) fields

2.1 Epidemiological studies

An interesting observation regarding ELF-MF exposure from in-built transformers and possible increased risks of melanoma and ALL for childhood exposure required follow-up. The question whether magnetic fields or shocks were underlying the previously observed associations with ALS or motor neuron disease remained unanswered.

2.1.1 Childhood cancer

Nguyen et al. [21] followed up on an earlier observation that residential proximity to plant nurseries was associated with risk of childhood leukaemia, possibly associated to elevated pesticide exposure (Nguyen EnvRes 2021 [22]). In California the space beneath powerlines is a preferred location for plant nurseries and Nguyen et al. (2022) investigated if this association could confound the observed association of powerlines and childhood leukaemia. The initial sample consisted of 5788 cases diagnosed with childhood leukaemia in California from 1986 to 2008 and 5788 age and gender matched controls. Due to imprecise geolocation of birth address, around 16% of case and controls were excluded, and data were analysed in conditional logistic regression model with adjustment for age, sex, socioeconomic status and ethnicity. Birth address proximity to powerlines was assessed from GIS-data and satellite imagery and categorized with cut points at 50, 150 and 300 m. For residences close enough to powerlines to potentially have elevated magnetic fields, field strength was calculated from information on distance, phasing and historical load and categorized with cut points 0.4, 0.2 and 0.1 µT. Plant nurseries were identified from government databases and confirmed from satellite imagery. Distance to nearest nursery perimeter was categorized with cut points at 75, 150, 300 and 600m. Living within 75m of a nursery was associated with OR 2.40 (95%CI: 0.99-5.82) for leukaemia, no elevated risk was observed at further distances. Comparing highest and lowest exposure categories, both distance to powerlines (OR: 1.47, 95%CI: 0.76-3.23) and magnetic fields (OR: 1.51, 95%CI: 0.70-3.23) were in line with previous observed risk estimates of leukaemia. Adjustment for nursery proximity only attenuated estimates very slightly to 1.38 (95%CI: 0.71-2.69) and 1.42 (95%CI: 0.65-3.16) respectively. Results were robust in sensitivity analysis but only 4 cases and 1 control had high exposure to both power lines and plant nurseries. The authors conclude that their results do not support plant nurseries as a strong confounder for the observation that children living close to powerlines may be at increased risk of leukaemia.
The very low number of children with relevant exposure to both powerlines and nurseries is a major limitation as noted by the authors.

Brabant et al. [23] published a systematic review and meta-analysis of case-control and cohort studies on magnetic fields from powerlines and electric appliances and risk of leukaemia in children and young adults (<21 years of age). For powerlines they combined studies assessing magnetic flux density, distance to powerlines or wire coding exposure to powerlines using dichotomized exposure with cut points of 0.2µT, 200m, and low vs high current configuration. In this analysis of 21 studies comparing exposure above and below the cut points, OR for childhood leukaemia was 1.26 (95%CI 1.06-1.49). In a meta-analysis based only on magnetic field strength only exposure above 0.4µT was associated with increased risk of childhood leukaemia (12 studies, OR=1.37, 95%CI: 1.05-1.80).

Living within 50m of a power line was associated with OR 1.07 (5 studies, 95%CI: 0.87-1.30,). Living with high current configuration wire code was associated with OR 1.23 (5 studies, 95%CI: 0.72-2.10). Higher risk estimates were observed for acute lymphoblastic leukaemia and for studies published before year 2000 (OR: 1.51, 95%CI: 1.26-1.80, n=11) than for later studies (OR: 1.04, 95%CI: 0.84-1.29, n=10) .
Based on four studies they also analysed risk for childhood leukaemia associated with electrical blankets (OR: 2.75, 95%CI: 1.71-4.42), electrical clocks (OR: 1.27, 95%CI1.01-1.60), water beds (OR: 1.11, 95%CI: 0.85-1.47), and hair dryers (OR: 1.40, 95%CI: 0.79-2.48).
The overall association of childhood leukaemia with powerlines agrees with previous studies. The lower risk in more recent studies has also been reported previously but it remains unresolved if this is a true decrease. The meta-analysis of electrical appliances is based on four case-control studies published in 1998 or earlier, all based on self-reported data, and this may have introduced bias. This could be the case for example if early symptoms of leukaemia increases use of electrical blankets or if cases recalls past use of appliances differently from controls.

2.1.2 Adult cancer

Jalilian et al. [24] investigated occupational exposure ELF-MF and electricals shock in relation to risk for malignant lymphoma by means of job exposure matrices. They analysed a case control study nested in a census-based cohort covering Iceland, Norway Sweden and Finland with one or more time-points of information regarding occupation in the period 1960-1990. The follow up period was until 2005 for non-Hodgkin's lymphoma (n=68978), chronic lymphocytic leukaemia (n=20615) and multiple myeloma (n=35467). Cases were extracted from national cancer registers. For each case, five controls were matched on country, year of birth and sex. Cases and controls where required to be 20+ years at time of diagnosis and to have provided occupational information in at least one census. Job exposure matrices (JEM) were used to classify occupational exposure levels to ELF-MF and electrical shocks into background, low and high. Assigning values of 1, 2 and 4 to these categories and multiplying with number of years in occupation, cumulative unit years were calculated. Logistic regression with adjustment for social class and occupational exposure to solvents was used to evaluate these and highest level of exposure ever as risk factors for the three lymphoma types. All observed ORs were very close to unity, none were statistically significantly elevated, but many were significantly lower than the reference. The results were robust in a range of sensitivity analyses. The authors conclude that their results do not support an association between occupational exposure to ELF-MF or electric shocks and lymphoma.
The prospective data collection, large sample size and register based case identification are strengths of the study. The reliance on self-reported occupations, collected in intervals of 10 or more years and the reliance on job exposure matrices makes some exposure misclassification inevitable. Additionally, there was some indication that higher exposure might be associated with some healthy worker effect. These factors may have limited the study's power to detect detrimental effects.

2.1.3 Symptoms

In a 15-story office building in Israel, which was situated 10 m from a 161 KV overhead powerline, Raz-Steinkrycer et al. [25] conducted a cross sectional study on ELF-MF exposure and symptoms. Exposure for each floor was assessed on separate days by placing 40 dataloggers on office desks to cover the entire floor. The dataloggers recorded field levels every minute, and exposure was calculated as the average of all measurements between 7.a.m and 7.p.m. Perceived ELF-MF exposure and 26 different health symptoms were assessed by questionnaire which also covered lifestyle, socioeconomic information and working conditions. A total of 487 employees (81% of those invited) participated. Average daily exposure level per workplace ranged from to 0-0.6 µT. Perceived exposure (Categories

from 0=low to 4=high) was not correlated with measured exposure (r=0.06). In logistic regression analysis, adjusting for age, gender, sociodemographic characteristics, smoking, years of working in building and average daily work hours, measured exposure was significantly associated with increased reporting of “exhaustion and weakness”, “frustration/worry” and “headache”. These three outcomes were also associated with perceived exposure. In addition, perceived exposure was also associated with “dyspnoea”, “eye pain or irritation” and “sleepiness”. The authors conclude that working near high voltage powerlines appears to produce both physiological and psychological effects.
Strength of the study were the measurements performed at each workplace. Weaknesses include that exposure for different floors was measured on separate days which may have reduced precision of exposure assessment. It is puzzling that even though perceived and actual exposure were not correlated, both were associated with the same few health endpoints. No additional analysis was presented to understand this occurrence and no data were provided on the number of participants with high perceived or measured exposure. Measured and perceived exposure was associated with proximity to the power line and therefore with certain parts of the building which may have introduced apparent associations if people with symptoms for other reason are more likely to work here.

2.1.4 Other outcomes

Touitou et al. [26] performed an analysis of serum cortisol in relation to ELF-MF from a study, where collected data on other blood parameters were already published in 2013 (Touitou et al, Clinical Biochemistry 2013). Twenty-nine men aged 30 to 48 years were included into the study. Fourteen exposed workers were occupationally as well as residentially exposed, as they were working for the high voltage transmission system in Paris and had their homes close to the substation where they worked. Fifteen unexposed workers were matched to be similar in age, size, physical activity levels and circadian rhythm. All participants wore an Emdex (ELF magnetic field meter) for a week, which measured ELF-MF every 30 seconds. All participants stayed in a clinic overnight for 12 hours (8 pm to 8 am), during which time 13 blood samples were taken, and hourly serum cortisol levels were determined. Arithmetic mean field levels of unexposed was 0.04 µT and of exposed was 0.72 µT. Based on a significant interaction (ANOVA) between exposure status and time, the authors state that ELF-MF exposure decreased the peak-time serum cortisol levels, although the general secretory pattern of cortisol was unaffected.
The authors do not report in which year the study was actually performed. The previous analysis on the same participants on haematological and immune system functions did not reveal differences among exposed compared to unexposed workers. It remains somewhat unclear if the measurements were taken before, during or after the blood measurements and if there were remaining differences between the exposed and unexposed groups of workers that could possibly explain the subtle differences in cortisol timing between the groups. Given the small sample size, chance might also be an explanation for the observed interaction. Neither this, nor the original publication provided an overview of the sociodemographics of the groups, which could have helped to understand this issue.

2.1.5 Conclusions on ELF epidemiological studies

Leukaemia has been quite consistently associated with childhood leukaemia. The evaluated studies do not allow conclusions as to whether the observed association is causal. Residential proximity to plant nurseries with presumably higher pesticide exposure levels did not act as a strong confounder.
ALS or motor neuron disease has been associated with magnetic fields and with electric shocks. The two exposures are correlated and studies summarized this year do not allow conclusions regarding what drives the observed association.
A large study on occupational exposure ELF-MF and electricals shock in relation to risk non-Hodgkin’s lymphoma, chronic lymphocytic leukaemia and multiple myeloma did not observe

increased risks but exposure misclassification and health worker effects may have affected study results.

Table 2.1: Epidemiological studies on exposure to ELF fields

Endpoints	Reference	Exposure assessment	Study design, Population	Results
Childhood Leukemia	Brabant et al 2022	Exposure from powerlines assessed with different methods combined by dichotomization of exposure. Self-reported exposure from electrical appliances.	Meta-analysis of 21 studies.	High exposure from power lines associated with elevated risk of childhood leukaemia. Most pronounced for studies published before year 2000.
Childhood Leukemia	Nguyen et al 2022	Birth address magnetic field strength, established from power lines established from GIS-data, satellite imagery, phasing and power load. Proximity to plant nurseries from administrative databases and satellite images	Case-control, 5788 cases and 5788 controls from California, USA, 1968-2008	Study assessed if confounding by pesticide could explain previously observed association between exposure to magnetic fields from power lines and leukemia. Plant nursery proximity and associated pesticide exposure (more common under power lines) did not appear as a strong confounder.
Malignant lymphoma	Jalilian et al 2022	Occupational exposure to ELF-MF and electric shocks established from census data and JEMs	Case-control, NHL cases: 68978, Chronic Lymphocytic Leukaemia: 20615, Multiple Myeloma: 35467. 5 matched controls per case. Norway, Sweden, Finland, and Iceland. 1960-2005	There was no evidence of an association of ELF-MF or electric shock exposure with malignant lymphoma in this large register-based study.
Serum cortisol levels	Touitou et al, 2022	Residential and occupational exposure, measured week-long with Emdex	High voltage power line workers, N=29 men aged 30-48, of which 14 exposed and 15 unexposed.	General secretory pattern of cortisol unaffected among exposed, peak-time serum cortisol levels decreased.
Self-reported symptoms	Raz-Steinkrycer et al 2022	Perceived and measured daily exposure to ELF-MF from a powerline next to an office building	Cross sectional study, 487 employees participated. Tel-Aviv, Israel	Self-reports of "exhaustion and weakness", "frustration/worry" and "headache" were all associated both with perceived and measured exposure, which were however not associated with each other. Alternative explanations for the observed associations were not explored.

2.2 Human studies

While the topic phosphene perception, which was studied in 2021 was not investigated in 2022, the topic perception thresholds of AC and DC electric fields was pursued in 2022 by another investigation from the same group. Jankowiak et al. [6] followed-up the aspect of a reduced perception threshold for electric fields in hybrid AC and DC exposure conditions. Two other human experimental studies published in 2022 addressed effects of ELF exposure. Legros et al. [27] Investigated whether it is feasible to analyse hormone concentration in samples of blood from the fingertip Since this study did not provide results on ELF effect, it was not further discussed. Another study from the same group investigated whether extremely low-frequency magnetic and electric field stimulation of the vestibular system has effects on human subjective visual vertical perception (Bouisset et al. [28]).

Bouisset et al. [28] investigated effects of vestibular ELF magnetic and electric stimulation on subjective visual vertical perception (SVV). The SVV is a vestibular-specific test, it is a measure of the angle between perceived vertical and "true" (gravitational) vertical. The line was always oriented towards the left with a random starting angle between -25 and -20°. Participants were asked to control the angle with a mouse so that the white line aligns with what they perceived to be the gravitational vertical. Thirty-three healthy volunteers (10 males and 23 females, age: mean ± SD: 24.6 ± 4 years) participated in the study. ELF-MF was delivered by a single-coil centred at the left mastoid process with an integrated water-cooling to dissipate the heat generated by the system. To further favour participant's blinding, coil vibrations and sound production were minimized. The coil was powered by an MTS magnetic resonance (MRI) gradient amplifier array capable to deliver up to 200 A_{rms} at ± 345V. DC and AC stimulation were produced with a transcranial current stimulation device. The DC stimulation was used as a positive control since DC is known to, on average, bias SVV towards the anodal side of the stimulation. Subjects were exposed in a single session of approximately 1.5 h duration in a double-blind randomized cross-over design to (i) control trials with no stimulation, (ii) DC at 2 mA used as a positive control, (iii) AC stimulations sinusoidal, peak ±2 mA) given at four frequencies (20, 60, 120, and 160 Hz), and (iv) alternating sinusoidal ELF-MF stimulations at the same frequencies. Results indicate that for similar levels of SVV precision, the ELF-MF condition required more time to adjust SVV, and SVV variability was higher with ELF-MF than with AC vestibular-specific stimulations. Yet, the differences between AC and ELF-MF stimulations were small. Overall, this study highlights small differences between AC and ELF-MF vestibular stimulations. According to the authors the study results have implications for international exposure guidelines and standards.

In 2021 Kursawe et al. [29] reported that co-exposure of DC and AC electric fields lead to a decrease of the perception threshold compared to either AC or DC exposure alone (SSM last year's report [1]). Due to the relevance of hybrid EF perception for the approval procedures of hybrid power lines in the proximity of residential areas, Jankowiak et al. [6] pursued this finding to investigate the influence of alternating currents (AC) on the whole-body detection thresholds of hybrid EFs from AC and direct currents (DC) and to explore the lower bound of human hybrid EF perception. They contacted healthy participants from the earlier study (Kursawe et al. 2021), who were able to detect hybrid EF strength combinations of at least 5 kV/m AC and 8 kV/m DC. 92 subjects fulfilled this criterion, of those 51 (20 men and 31 women, age range: 23 to 77 years, mean ± SD: 49.14 ± 17.52 years) agreed to participate in the actual study. The experiments were carried out in a specially designed exposure laboratory, described in detail by Jankowiak et al. (2021) . The method used for EF threshold detection 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). An exposure trial could either be detected correctly (hit) or was not perceived (miss). In line with this, sham trials could be classified as falsely perceived (false alarm) or correctly denied (correct rejection). From these numbers, sensitivity (d') towards a given EF was individually calculated. Values of d' ≥ 1 indicate a successful detection performance. Four different test conditions were designed with constant AC EF strengths of 1, 2, 3, or 4 kV/m (rms values) at a frequency of 50 Hz combined with varying DC EF strengths of 1, 2, 4, 8, or 16 kV/m leading to various total EF strengths (see table 2.2). Each test condition, which was randomly assigned and lasted approximately 15 min and consisted of 40 trials (sham and real exposure 20 trials each) with randomized trial sequence. For each participant all eight resulting test sessions were performed on a single test day in a double-blind design. Temperature (22 °C) and humidity (50%) were constant during testing. The results indicate that very low combinations of AC and DC (see exposure categories and results in Table below) were reliably perceived by at least one participant. Detection thresholds were significantly lower with increased AC EF strengths, which underlines the role of AC in the human perception of hybrid EFs.

Table 2.2.1: Number of participants who reliably perceived the exposure by AC, DC and total EF field strength.

AC (kV/m)	Total EF strength (kV/m)					# of participants with reliable perception (N=51)				
	DC (kV/m)					DC (kV/m)				
	1	2	4	8	16	1	2	4	8	16
1	1.41	2.24	4.12	8.06	16.03	1	3	2	14	30
2	2.24	2.83	4.47	8.25	16.12	4	3	6	29	42
3	3.16	3.61	5.00	8.54	16.28	9	7	14	29	42
4	4.12	4.47	5.66	8.04	16.49	11	13	25	35	43

As stated in last year's report: Since perception of EF is not yet an adverse health effect as such, 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 recommendations for hybrid exposure.

2.2.1 Conclusion Human studies

Studies published in the current reporting period revealed, a) that in an ELF-MF exposure condition more time was needed to reach a specific level of precision in the subjective visual vertical perception as compared to an AC stimulation although the differences were small and b) very low combinations of AC and DC electric fields (1 kV/m each) were reliably perceived by at least one participant. Detection thresholds were significantly lower with increased AC EF strengths, which underline the role of AC in the human perception of hybrid EFs.

Table 2.2.2: Human studies on exposure to ELF fields

Endpoints	Reference	Exposure condition	Sample	Results
subjective visual vertical perception	Bouisset et al. (2022)	(i) control trials with no stimulation, (ii) DC at 2 mA used as a positive control, (iii) AC stimulations sinusoidal, peak ±2 mA) given at four frequencies (20, 60, 120, and 160 Hz), and (iv) alternating sinusoidal ELF-MF stimulations at the same frequencies; duration of exposure: 30s per experimental condition	33 healthy volunteers (10 males and 23 females, age: mean ± SD: 24.6 ± 4 years)	Results indicate that for similar levels of SVV precision, the ELF-MF condition required more time to adjust SVV, and SVV variability was higher with ELF-MF than with AC vestibular-specific stimulations. Yet, the differences between AC and ELF-MF stimulations were small.
Perception of hybrid AC and DC exposure	Jankowiak et al. (2022)	Condition 1: AC EF strength 1 kV/m in combination with DC EF strength 1, 2, 4, 8, 16 kV/m. In conditions 2 to 4 an AC EF of 2, 3, and 4 kV/m were combined with the DC EF fields.	51 (20 men and 31 women, age range: 23 to 77 years, mean ± SD: 49.14 ± 17.52 years)	Very low combinations of AC and DC electric fields were reliably perceived by at least one participant. Detection thresholds were significantly lower with increased AC EF strengths, which underline the

				role of AC in the human perception of hybrid EFs.

2.3 Animal studies

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

For the reporting period 2022, nine rodent studies were identified, all in rats; in addition to these, also three studies on non-mammalians described effects of extremely low frequency magnetic fields on honeybees and bivalve mollusc.

2.3.1 Cognition and Behaviour

Acikgoz et al. [30] investigated the effects of the exposure to extremely low-frequency magnetic field (ELF-MF) and zinc supplementation during the prenatal and postnatal periods on behavior and synaptic protein in a gender dependent manner. Pups from four groups of pregnant rats were used: Sham (39 pups), ELF-MF (39 pups; 5 days/wk, 4 h/day exposure applied), Sham+Zinc (29 pups; 5 days/wk, 5 mg/kg/day zinc applied) and EMF+Zinc (27 pups; 5 days/wk, 4 h/day ELF-MF-exposure and 5 mg/kg/day zinc applied). They exposed pups to 50-Hz, 3-mT ELF-MF, produced by a Helmholtz coil system, from the first day of pregnancy (exposing dams) to the 42nd postnatal day. Zinc was administered by gavage till the end of the pregnancy. Behavioural experiments, consisting in Crawley's sociability test, open field test and elevated plus maze test, were performed on day 42; blood and brain tissue samples were taken and evaluated.
ELF-MF exposure caused a reduction in the number of pups per birth. Behavioural tests showed that ELF-MF exposure had no effect on social behaviour, but adversely affected activity and exploratory behaviour, and led to increased anxiety formation.

Hosseini et al. [31] exposed 4 groups of 3-month-old female Wistar rats (n=6 per group) to 50 Hz, 100 μT ELF-MF, for 21 days, 4 h/day, alone or combined with chronic stress. The stress period was induced for 3 weeks before and 3 week of pregnancy using various methods. Female offspring were selected for behavioural tests (elevated plus-maze, open field test) that were performed at the age of 40 days. The authors observed that anxiety-like behaviour increased in all treatment groups and ELF-MF increased anxiety induced by maternal stress in the ELF-MF/Stress group. The stress group showed decreased serotonin and increased corticosterone levels. ELF-MF elevated the PNMDAr2/NMDAr2 ratio and 24(S)-hydroxy cholesterol compared to the control group but did not change corticosterone.

2.3.2 Development

Ersoy et al. [32] performed a study to clarify the effects of prenatal and postnatal ELF-MF exposure on testis development. They exposed pups from three groups of Sprague-Dawley pregnant rats (n=3 for sham, n=6 other groups). The mothers were exposed during the entire pregnancy to 50 Hz, 3 mT ELF-MF (5 days/wk, 4 h/day), produced with a Helmholtz coil pair system. Litters were further exposed until postnatal day 28 and divided into two groups on day 28. One group continued to be further exposed to ELF-MF until the 42nd day. The second group was kept without exposure until the 42nd day. Testis tissues and blood samples of male offspring were collected. The analysis showed that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were decreased in animals exposed 42 days. Decreased SHANK3 and SH3 protein and antioxidant levels were also reported in the group exposed 28 days. A deterioration in the structure and function of the testis and a decrease in growth factors (VEGF, IGF1) that would affect testicular functions were observed, showing that ELF-MF exposure during embryonic development and adolescence can cause apoptosis and structural changes in the testis.

Ozturk et al. [33] exposed pups (n=14 for the control group, n=12 for the exposed group) from two groups of Sprague-Dawley pregnant rats, to 50-Hz, 500 μT ELF-MF (produced by a Merritt coil system), 24 h/day from day 0 of gestation to day 21 of lactation. They investigated the potential effects of ELF-MF exposure during the gestational and lactational period of dams on immune system parameters. For this purpose, at sacrifice, a blood sample and the spleen of the animal were collected and subjected to analysis. Significant changes were found in white blood cells (WBC) and lymphocyte counts. CD4+ cells were significantly increased in the female group exposed to ELF-MF. A significant increase in Th cells was found in the comparison between female exposed group and female control group, although proliferation Th, Tc, NK and B cells did not change between prenatal and postnatal exposure group and control group. Also, IL-17A and IFN-γ levels significantly increased in plasma and spleen and the expression of the IL-17A gene was upregulated resulting in CD4+ cell proliferation and inflammation. The mean IL-4 level and the expression level of the IL-4 gene were not changed.

2.3.3 Oxidative stress

Guleken et al. [34] exposed pups from Sprague-Dawley pregnant rats: after confirmation of gestation, female rats were randomly divided into two groups: the control group, which was not exposed to ELF-MF during the experiment, and the electromagnetic field group that had prenatal and postnatal ELF-MF exposure. The control group consisted of n = 14 offspring who remained under normal conditions, the ELF-MF group consisted of n = 12 offspring that were exposed continuously (24 h/day) to a 50 Hz ELF-MF at field strength of 500 μT from day 0 of gestation through to day 21 of lactation. To expose animals, a Merritt coil system, which consists of four-square coils connected in series, was used. They measured malondialdehyde (MDA) and glutathione (GSH) levels, and observed that lipid peroxidation was increased and the antioxidant response was decreased. Authors concluded that ELF-MF exposure at 50 Hz may result in an imbalance between oxidative and antioxidative blood and plasma parameters of rat offspring. To identify the chemical changes, they collected Raman spectra of cerebellum, left brain, right brain and liver tissue from the control and exposed groups. In the brain samples the shift of peaks corresponding to the amide III vibrations existed after ELF-MF exposure. Structural changes were detected in CH2 vibrations originating from lipids in both hemispheres. Additionally, the number of amide III bonds was increased with ELF-MF exposure in the cerebellum and left-brain tissue. In liver tissue higher Raman intensities were visible in the tissues from the ELF-MF group. In this group ELF-MF exposure also caused structural changes in lipids. Principal component analysis (PCA) indicated that it was possible to distinguish ELF-MF and control groups. Consequently, hierarchical component analysis (HCA) showed that tissues from ELF-MF and control groups separately created similarity with the groups.

Klimek et al. [35] evaluated changes in the levels of oxidative stress and antioxidant defense markers in the prefrontal cortex of adult rats after repeated exposure to ELF-MF and assess whether repeated ELF-MF exposure can modify oxidative/antioxidative status in response to other stress factors. They used 3-month-old male Wistar rats (n=175) divided into six groups: (1) animals exposed to EMF (50 Hz, 1 mT), in which the basal level of markers was assessed; (2) animals exposed to EMF (50 Hz, 1 mT) and exposed to open field test; (3) animals exposed to ELF-MF (50 Hz, 7 mT) in which the basal level of markers was assessed; (4) animals exposed to ELF-MF (50 Hz, 7 mT) and exposed to open

field test; (5) control animals subjected to the same experimental procedure as the experimental groups 1 and 3, except ELF-MF exposure; (6) control animals subjected to the same experimental procedure as the experimental groups 2 and 4, except ELF-MF exposure. Exposure to “low” (1 mT) and “high” (7 mT) ELF-MF was performed in three different periods. Each period included 7-day exposure, 1 h/day. The experimental design included two sets of experiments. During the first set of experiments, a part of rats (n = 88) after each period of ELF-MF or control exposure was sacrificed to estimate the effect of ELF-MF on oxidant/antioxidant status (8-isoprostanes (8-epi PGF2α), protein carbonyl groups, and level of total antioxidant capacity). Remaining animals (n=87), after ELF-MF treatment, were exposed to another stress factor, the open field.
Results showed that changes in the oxidative/antioxidative status after 1 mT ELF-MF were enough to reduce, and after 7 mT ELF-MF to intensify oxidative processes in response to the next stress. They concluded that the organism might adapt to “low” ELF-MF, (by activation of intrinsic signaling pathways directed into the decrease of oxidative stress), while “high” ELF-MF exceeds the adaptive capacity of the organism and sensitizes it to subsequent stress, and thus may modulate vulnerability to diseases mainly related to the nervous system.

2.3.4 Other endpoints

Aydinbelge-Dizdar et al. [36] studied 4 groups of 3-5 month-old female Wistar rats (n=8 for sham, n=9 other groups). They exposed 3 groups of animals to ELF-MFs of 1, 1.5, and 2 mT emitted by 3 Helmholtz coils 4 h/day for 30 days, to investigate the effect of exposure to ELF-MF on nasal mucociliary clearance by rhinoscintigrapic and histopathological evaluation.
Rhinoscintigraphy was performed to measure nasal mucociliary clearance. Nasal mucociliary clearance rates (NMCR) was decreased with increasing ELF-MFs, in 1.5 and 2 mT groups compared to control. Nasal mucociliary transport rate (NMTR) was decreased in the groups exposed to 1.5 and 2 mT ($p < 0.05$) compared to control. The edema, hyperemia, inflammation, ciliary loss, and goblet cell density were statistically significant different between control and groups exposed to 1.5 and 2 mT. The authors concluded that exposure to ELF-MF higher than or equal to 1.5 mT was detrimental to nasal mucosa mucociliary function, depending on the intensity of the ELF-MF.

Sert et al. (Sert et al., 2022) [37] studied 8 month old, female Wistar rats, divided into 3 groups (n=12 per group). Two experimental groups were exposed to a 50 Hz ELF-MF field of 0.4 mT for 6 h/day for 5 days or 10 days, depending on the group. The field was generated by a device designed by the researchers that had two pairs of Helmholtz coils. The control group was kept in the same environment, without any exposure to a magnetic field. At the end of the exposure, blood collection from the rats and laboratories analysis were performed. The authors found a significant increase in the Fetuin-A values and in glucose, HbA1c, and Hba1 IFCC values in both of the experimental groups compared to the control group. A decrease was observed in Fetuin-A, HbA1c, HbA1c IFCC and glucose values to 10 days of ELF-MF application compared to 5 days of application and values were closer to normal in the 10-day application.

Vornoli et al. [38] studied the evolution of toxicant-associated fatty liver disease, preneoplastic and neoplastic lesions of the liver and the potential enhancing effect of lifespan exposure to an 50 Hz ELF-MF in Sprague-Dawley rats treated with Aflatoxin B1 (AFB1). Two groups of male and female SD rats, consisting of 427 animals in total, were treated with 70 mg/rat AFB1. Of these rats, one group of 222 animals had also been exposed to 50 Hz, 1000 mT ELF-MF. Animals were exposed lifespan, since embryonic life (12th day of pregnancy), irradiating the female breeders, 19 h/day. A total of 215 animals constituted the control group. Ten rats/sex/group were subjected to 8 interim sacrifices between 10 and 80 weeks of age, while the remaining animals were sacrificed at 118 weeks of age.

Histopathological analysis was perfomed on samples collected after animals death.
Authors didn't observed any enhancing or toxic effect in the liver of rats exposed to ELF-MF.

2.3.5 Studies in non-mammalians

Migdał et al. [39] evaluated the effect of an anthropogenic electromagnetic field environmental pollution, due to electric infrastructure components such as transformers and power lines, on honeybee navigation. One-day-old worker honeybees (*Apis mellifera* L.) were exposed to a 50 Hz magnetic field generated in a solenoid. They applied three different exposure durations (10 min, 1 h, and 3 h) and two magnetic field intensities (1 mT and 1.7 mT) for each exposure duration, resulting in a total of 6 experimental groups and 1 control group (n=1000 per group). Authors evaluated the effect of ELF-MF on behaviour. Sixty randomly chosen bees from each group were taken to a glass container and filmed. During the whole experiment, 420 worker bees were evaluated for seven types of behaviour (walking, flight, body cleaning, contact between individuals, wing movement, stillness and loss of balance). Exposed animals showed differences in behavioral patterns and loss of balance. Loss of balance indicate a disturbance of the honeybee by the magnetic field.

Li et al. [40] also performed a honeybee study, in this case *Apis cerana*. They wanted to evaluate the effect of ELF-MF on the development of bee larvae. The authors transferred 2-days-old honeybee larvae on 24-well tissue culture plates, divided into 2 groups (n=72 larvae per group): three plates were not exposed (control group), the other three plates were put into the ELF-MF generator and exposed to 50-Hz, 3 mT ELF-MF from the second day of life to the end of individual development. Significant decreases of the survival rate and of the body weight, significant extended duration of development time and interference with the process of metamorphosis and pupation after ELF-MF exposure were reported. The transcriptome sequencing also showed that ELF-MF exposure decreased nutrient and energy metabolism, impeded degradation of larvae tissues and the rebuilding of pupae tissues in the metamorphosis process, and interfered with the growth and development of honeybee larvae.

Jakubowska-Lehrmann et al. [41] performed two experiments to evaluate the effects of both static magnetic field (SMF) and ELF-MF, to simulate the exposure generated by submarine cables. Both studies were carried out in an analogous way. SMF or ELF-MF generators (depending on the experiment), consisting of Helmholtz coils, were used to expose bivalve *Cerastoderma glaucum* in experimental aquaria. Bivalves were divided in a group exposed to SMF or ELF-MF of 6.4 mT for 8 days and a control group (n=30 per group for SMF exposure; n=33 per group for ELF-MF exposure). The authors investigated potential effects on oxygen consumption, ammonia excretion, bioenergetics, oxidative stress, and neurotoxicity. Results showed that bivalves maintained a positive energy balance, but the filtration rate and energy available for individual production were significantly lower in SMF-exposed animals compared to the control. No changes in respiration were reported, but ammonia excretion rate was significantly lower after exposure to ELF-MF. Acetylcholinesterase activity was inhibited.

2.3.6 Summary and conclusions on ELF animal studies

Nine studies in rats were examined this year. Also, two studies on honeybees and one study on molluscs were identified.The rat studies indicated different adverse effects, in particular related to behavioural effects, development and oxidative stress.

Even if some behavioural aspects have not been modified by exposure to extremely low frequency magnetic fields, such as social activity, an increase of anxiety-like behaviour has been observed, in two different works and in line with other studies identified last year. In addition, an adverse effect on activity and exploratory behaviour of ELF-MF exposure has been shown.

Other studies examined the effect of ELF-MF exposure on nasal mucociliary clearance and the onset of diabetes, respectively. The first study found that edema, hyperemia, inflammation, ciliary loss, and goblet cell density showed statistically significant differences between control and exposed groups and that the nasal mucociliary clearance rate decreased with increasing ELF-MFs. The second work showed an increase in Fetuin-A, proposing that diabetes can be caused by increased insulin resistance determined by Fetuin-A level.

Studies applying prenatal and postnatal exposure reported ELF-MF effects on the development of the reproductive and immune system. A deterioration in the structure and function of testis and decrease in growing factors (VEGF, IGF1), which could affect testicular functions, were observed. Regarding immunity, various differences were observed between exposed animals and control groups. Significant changes were found in WBC and lymphocyte counts. Also, IL-17A and IFN-γ levels significantly increased in plasma and spleen.

Regarding oxidative stress, authors of different studies have found a decrease in oxidative processes after treatment with 0.5-1 mT ELF-MF. An increase of the oxidative stress after 7 mT ELF-MF treatment has also been reported. In this case it is suggested that an ELF-MF so "high" exceeds the adaptive capacity of the organism.

A long-term study on the possible enhancing or toxic effects in the liver of rats treated with Aflatoxin B1, after exposure to ELF-MF has also been performed and authors didn't observe any significant change compared to the control group.

The two honeybee studies showed effects both at the level of larval development and at the level of behaviour. Larvae showed higher mortality and slower development, accompanied by decreased body weight, metabolic problems and interferences in the metamorphosis phases. Behaviour was found to be affected by exposure to ELF-MF, honeybees showed differences in behavioural patterns (walking, flight, body cleaning, contact between individuals, wings movement, stillness and loss of balance were examined) with the occurrence of a peculiar symptom (the loss of balance). These data are in line with findings of the previous year by the same group of researchers.

Finally, both oxidative stress and neurotoxicity effects were observed on bivalve molluscs after exposure to an ELF-MF field, where a decrease in ammonia excretion rate was reported.

Table 2.3: Animal studies on exposure to ELF fields

Endpoint	Reference	Exposure conditions	Species, strain, sex	Results
Rodent studies				
Cognition and behaviour	Acikgoz et al. (2022)	50 Hz, 3 mT 4 h/d, 5 d/wk prenatal and 42 days postnatal	Rat, Sprague-Dawley	SHANK3 and NLGN3 proteins significantly lower in ELF groups ELF may alters the levels of synaptic proteins in the developing brain, leading to behavioural changes in a gender-dependent manner.
	Hosseini et al. (2022)	50 Hz, 100 µT 4 h/d, 21 d	Rat, Wistar, female	Anxiety-like behaviour increased. PNMDAr2/NMDAr2 ratio and 24(S)-hydroxy

				cholesterol increased. Corticosterone not changed.
Development	Ersoy et al. (2022)	50 Hz, 3 mT 4 h/d, 5 d/wk Prenatal and postnatal (28-42 days)	Rat, Sprague-Dawley	Decreased SHANK3, VEGF, and IGF1 protein levels, deterioration in the structure and function of testis
	Ozturk et al. (2022)	50 Hz, 500 µT 24 h/d From day 0 of gestation to day 21 of lactation	Rat, Sprague-Dawley	Significant changes in WBC and lymphocyte counts. CD4+ cells significantly increased in the female group exposed. IL-17A and IFN-γ levels increased in plasma and spleen, and IL-17A gene expression upregulated. Mean IL-4 level and expression level of IL-4 gene not changed.
Oxidative stress	Guluken et al. (2022)	50 Hz, 500 µT 24 h/d from day 0 of gestation to day 21 of lactation	Rat, Sprague-Dawley	Lipid peroxidation increased. Antioxidant response decreased. In brain samples a shift of peaks corresponding to the amide III vibrations and structural changes detected after ELFMF exposure in CH2 vibrations. Number of amide III bonds increased with ELFMF exposure in cerebellum and left-brain tissue. Higher Raman intensities visible in the ELFMF group, in liver.
	Klimek et al. (2022)	50 Hz, 1 mT and 7 mT 1 h/d 3times/d 7 day	Rat, Wistar, male	Increased oxidative stress after exposure to 7mT EMF, decreased after 1mT EMF exposure
Other endpoint	Aydinbelge-Dizdar et al. (2022)	1 mT, 1,5 mT and 7 mT 4 h/d 30 days	Rat, Wistar, male	Nasal mucociliary clearance rate decreased with increasing ELF-EMFs, in 1.5 and 2 mT groups. Nasal mucociliary transport rate decreased in the groups exposed to 1.5 and 2 mT. Edema, hyperemia, inflammation, ciliary loss, and goblet cell density showed statistically significant differences between control and exposed groups
	Sert et al. (2022)	50 Hz, 0,4 mT 1 h/d 5 or 10 days	Rat, Wistar, female	No significant difference between the groups in transaminases and lipid profiles and C-Peptide. No significant difference in insulin, urea, creatinine, Na, K, Ca, and uric acid parameters. Significant increase in the Fetuin-A, Glucose, HbA1c, and Hba1c values in the experimental groups compared to the control group.

	Vornoli et al. [42]	S-50 Hz 19 h/d lifespan (since 12th day of pregnancy)	Rat, Sprague-Dawley	No changes in evolution of toxicant-associated fatty liver disease, preneoplatic and neoplastic lesion of liver
Studies in Non-mammalians				
Cognition and behaviour	Migdal et al. (2022)	50 Hz, 1 mT and 1.7 mT 10 min, 1 h, 3 h	Honeybee *(Apis mellifera L.)*	Changes in behaviour. Exposed animals showed loss balance.
Development	Li et al. (2022)	50 Hz, 3 mT From 2nd day of life to end of development	Honeybee, larvae (*Apis cerana*)	Decreases of survival rate and body weight, extended duration of development time and interference with the process of metamorphosis and pupation. Decrease of nutrient and energy metabolism.
Oxidative stress	Jakubowska-Lehrmann et al. (2022)	6.4 mT 8 days	Bivalve (*Cerastoderma glaucum*)	Energy available for individual production significantly lower in SMF-exposed animals. Ammonia excretion rate significantly lower. Increased protein carbonylation but not changes in the activities of antioxidant enzymes and the lipid peroxidation. Acetylcholinesterase activity significantly inhibited

Abbreviations: bw: body weight; CD: cluster of differentiation; d: day(s); ELFMF: extreme low frequency magnetic field; EMF: electromagnetic field; HbA1c: glycater emoglobin; h: hour(s); IFCC: IFN-γ: interferon gamma; IGF1: insulin-like growth factor-1; IL: interleukin; min: minute; NLGN: neuroligin; NMDAr2: Nmethyl-D-aspartate receptor 2; PNMDAr2: phosphorylated N-methyl-D-aspartate receptor 2; SHANK: SH3 and multiple ankyrin repeat domains; SMF: static magnetic field; VEGF: vascular endothelial growth factor; WBC: white blood cells; wk: week(s).

2.4 Cell studies

Last year, all the effects of ELF-EMF described *in vitro* seemed to depend on the experimental conditions (cell types, exposure duration, frequency, field intensity).

Compared to the previous Council report a lower number of publications was identified. For the reporting year 2022, the *in vitro* studies were carried out on several cell types of human origin dealing with proliferation, DNA damages and oxidative stress. One paper focusing on the causal association between exposure to ELF and childhood leukaemia investigated whether exposure may be involved in human mesodermal cell differentiation into hematopoietic stem progenitor cells (HSPCs).

2.4.1 Proliferation

Elexpuru-Zabaleta et al. [43] evaluated the influence of different exposure duration (1, 4 , 8, 12, 20 and 24 h) to 0.1 mT or 1 mT ELF-MF on viability of three breast cell lines (MDA-MB-231, MCF-7, MCF-10A). Viability was assessed 48 h, 96 h and 192 h from start of ELF-MF exposure. The control group was placed in another incubator and subjected to the same procedures as experimental cells but without 50 Hz ELFMF exposure.
The data did not show statistically significant differences in all three breast cell lines after 48 h from start of ELF-MF exposure for all tested exposure conditions and cell types. 96 h after the start of exposure to 0.1 mT ELF-MF, the viability of MDA-MB-231 and of MCF-7 was increased after exposure duration ranging from 4 to 12 h and from 4 to 16 h, respectively (compared to the control non-exposed cells, n=3). Exposure to 1 mT ELF-MF produced an increase in viability in MDA-MB-231 (for exposures of 4 h and 8 h), in MCF-7 cells exposed for 1, 4, 8, 12, and 20 h and in MCF-10A cells, (for exposures of 12 and 16 h. For all three cell lines a decrease in viability was measured after an exposure of 24 h. After 192 h, no significant differences in the viability of any of the three breast cell lines were found between 0.1 or 1 mT ELF-MF-treated and control cells. Using trypan blue assay to count the number of live cells 96 h after exposure to 50 Hz ELF-MF for 4 h and 24 h, both MDA-MB-231 and MCF-7, showed an increase in the number of viable cells after exposure to 0.1 mT ELF-MF for 4 h compared with their respective unexposed controls (0 h). Both MCF-7 and MCF-10A breast cells exposed to 1.0 mT ELF-MF for 4 or 24 h showed a reduction in the number of cells compared to controls. Studies of the cell cycle distribution showed an influence of the ELF-MF exposure on cell cycle in the three cell lines. An increase of cell number in S phase was obtained in MDA-MB-231 cells after 4 h of exposure to 0.1 mT ELF-MF, and in MDA-MB-231 cells and in MCF- 10A with exposure to 1.0 mT ELF- MF of 4 and 24 h. Changes in the mitochondrial membrane potential of breast cell lines that can influence viability were also measured in MCF7 (0.1 mT) and MDA-MB-231 cells and in MCF- 10A at 1 mT (when compared to control non-exposed cells, n=3). ROS production was increased in all cell type with the exposures to 1.0 mT ELF-MF 4 h and 24 h after ELF-MF exposure. These results suggest that ELF-MF exposure influence the viability of breast cells in vitro.

In previous studies, Martínez et al. demonstrated that intermittent exposure to a weak, 50-Hz MF, in addition to increasing free radical levels in NB69 human neuroblastoma cells, promoted the activation of the MAPK-ERK1/2 and -p38 transduction pathways, as well as that of the EGF receptor. Such activations lead to a significant increase in the proliferation of NB69 cells (Martínez et al.,2016, 2019, 2021). In the present study, the same authors investigated whether in vitro exposure to a 50-Hz, 100-µT MF (30 to 120 min) can affect the expression of the oxidative-stress sensitive protein p53 (Martínez et al. [44]). The results revealed that MF exposure causes overexpression in both gene and protein expression of p53 (at 90 min of exposure, followed by underexpression at 120 min when compared to sham-exposed cells, n=3). Two conformational structures of p53 were specifically recognized by immunofluorescence: the functional folded wild type (wt) isoform and the unfolded p53 isoform. Both isoforms were present in control cells, with rates of 65.26±2.5 and 15.10±1.7% for wt p53 and unfolded p53, respectively. 120 min MF exposure induced underexpression of wt p53 (18.9±1.3% below the controls) and increased the expression of the unfolded isoform (54.1±6.3% above the controls) together with changes in its nuclear/cytoplasmic distribution. Additionally, MF exposure induced significant overexpression of the anti-apoptotic protein Bcl-2 if compared to sham-exposed cells after 60, 90 and 120 min of exposure.

Previously, Sun W.J. and collaborators found that L-type calcium channel and sphingosine kinase 1 (SK1) were involved in 50-Hz MF exposure-induced cell proliferation (Chen et al. 2020; Qiu et al. 2019; Yang et al. 2019). In a more recent paper, Ye et al. [45] investigated the role of intracellular Ca^{2+} and signal molecules related to sphingosine kinase 1 (SK1) in cell proliferation induced by 50-Hz MF in human amniotic epithelial (FL) cells. FL cells were exposed to a 0.4 mT 50-Hz MF for 1h with or without NIF (the inhibitor of L-type calcium channel) or BAPTA (a chelator of Ca^{2+}), and the cell proliferation was analysed 24 h after the different treatments (n= 6). Results showed that 50-Hz MF exposure promoted cell proliferation if compared to sham-exposed cells. The intracellular Ca^{2+} chelator, BAPTA, could completely inhibit 50-Hz MF-induced cell proliferation, whereas NIF, the inhibitor of L-type calcium channel, only partly blocked it, indicating that the intracellular Ca^{2+} was involved in MF-induced cell proliferation via pathways more than the L-type calcium channel. When cells were cultured in calcium-free medium, MF exposure also increased intracellular Ca^{2+} and promoted cell proliferation compared with the sham group, indicating that MF exposure could induce endogenous Ca^{2+} releasing to elevate intracellular Ca^{2+} and maintain cell proliferation. MF exposure also activated SK1 under the condition of calcium-free culture and MF-activated SK1 could be completely inhibited by BAPTA. MF-induced cell proliferation was abolished by SKI II, the specific inhibitor of SK1. MF exposure activated the Akt kinase if compared to sham-exposed conditions (n= 5) and treatment of FL cells with LY294002, the inhibitor of Akt, could delete the MF-induced SK1 activation under the condition of calcium-free medium (n= 4).

2.4.2 Other cellular endpoints (oxidative stress, DNA repair and cell differentiation)

Mustafa et al. [46] investigated the possible differences in cellular responses to 50 and 60 Hz magnetic fields (MFs) (100 μT MFs for 24 h) looking at reactive oxygen species (ROS) production, DNA damage, DNA damage repair rate, as well as gene expression related to oxidative stress and DNA damage signalling, alone or in combination with the chemical menadione (Mustafa et al., 2022). The reduction of menadione-induced ROS levels in cells exposed to 50 Hz fields was observed with all menadione treatment durations (30, 40, 50 and 60 min) if compared to sham-exposed cells (n=3-4). Several oxidative stress-related genes were found to be upregulated when quantified immediately at the end of MF exposure and before any menadione treatment. The upregulated genes were not consistently the same in the 50 and 60 Hz experiments (n=3). Differences in DNA damage-signalling gene expression were also marginal and consistent with the finding that no statistically significant effects were found on DNA damage level or DNA damage repair rate. The authors stated that while only weak effects were found on the endpoints measured, the results are consistent with MF effects on ROS signalling, but no firm evidence was shown for distinct frequency-dependent effects.

Takahashi & Furuya have established an *in vitro* protocol to simulate the differentiation of human mesodermal cells to hematopoietic stem progenitor cells (HSPCs) using human-induced pluripotent stem cells (hiPS) (Takahashi & Furuya, [47]). This cell model would be useful for focusing on the causal association between exposure to power-frequency magnetic fields (MFs) and childhood leukaemia. The differentiation of mesodermal cells into HSPCs was followed by flow cytometry and is characterized by the appearance of a population of CD34+CD38− cells, which is a human HSPC-enriched population and revealed that mesodermal cells differentiated to HSPCs. The team also detected the emergence of CD45dullCD34+CD38− cells (a highly enriched population of human hematopoietic stem cells). After a continuous exposure up to 300 mT of 50-Hz MFs for 7 days during the differentiation process, the percentage of emerged HSPCs from mesodermal cells was not statistically significant in exposed groups compared with those in the sham-exposed group ($n \geq 3$). These results suggest that exposure to 50-Hz MFs up to 300 mT does not affect the differentiation of human mesodermal cells to HSPCs, which may be involved in the initial process of leukemogenesis.

2.4.3 Conclusions on ELF cell studies

Five *in vitro* studies on ELF-EMF exposure were included in the report, which does not allow any firm conclusions regarding possible biological effects from exposure to ELF-MF. One more study was found but had to be excluded due to insufficient number of experiments.

Table 2.4: Cell studies on exposure to Extremely low frequency (ELF) fields

Cell type	Endpoint	Exposure conditions	Effect	References
MDA-MB-231 (Human Metastatic breast adenocarcinoma) MCF-7 (Human Metastatic breast adenocarcinoma with oestrogen-receptors) MCF-10A (Human breast epithelial cell line) n=3	Proliferation	1, 4, 8, 12, 16, 20, 24 h ELF-MF (0.1 or 1 mT)	Viability increase depending on the cell type and exposure duration. Decrease in viability after an exposure of 24 h.	Elexpuru-Zabaleta et al., 2022
NB9 Human Neuroblastoma cells n=3	Proliferation	100-µT ELF-MF (30 to 120 min)	Overexpression in both gene and protein expression of p53 at 90 min of exposure, followed by underexpression at 120 min. Significant overexpression of the anti-apoptotic protein Bcl-2 (at 60, 90 and 120 min of exposure)	Martínez et al., 2022
SH-SY5Y Human neuroblastoma cells n= 3-4	Oxidative stress and DNA damage	100-µT 50-60 Hz (24 h) ± menadione	Reduction of menadione-induced ROS levels in cells exposed to 50 Hz fields (menadione treatment durations of 30, 40, 50 and 60 min No significant effects on DNA damage level or DNA damage repair rate.	Mustafa et al., 2022
Human Hematopoietic stem progenitor cells (HSPCs) n≥ 3	Differentiation	Up to 300 mT of 50-Hz 7 days	No effect on the differentiation of human mesodermal cells to HSPCs	Takahashi & Furuya, 2022
Human amniotic epithelial (FL) cells.	Proliferation Intracellular Ca^{2+}	0.4 mT 50-Hz MF for 1h	Endogenous Ca^{2+} release involved in	Ye et al., 2022

n = 6	50-Hz MF-induced cell proliferation via the Akt-SK1 signal cascade.

3. Intermediate frequency (IF) fields

3.1 Epidemiological studies

The previous report stated that given the very scarce scientific literature on exposure to IF-MF and possible health effects, no conclusions could be drawn. No studies were identified in this year's reporting period and therefore, the same conclusion applies this year.

3.2 Human studies

As for the previous reporting periods, no human experimental study investigating effects of EMF exposure in the intermediate frequency range was published.

3.3 Animal studies

In last year's report, in total two studies in mice did not result in adverse effects on behaviour and genotoxicity. One animal study was identified for the current reporting year 2022.

3.3.1 Fertility

Sundaram et al. [48] (2022) continuously exposed sexually mature (8-12 weeks-old) male Sprague Dawley rats (n=7 / group) to 150 kHz magnetic field (0.3 V/cm) or sham for 8 weeks, 24 hours/day (except for an hour dedicated to cleaning). The uniform field was generated by two flat electrodes made of aluminum foil-covered cardboard placed vertically at opposite ends of the cage. The duration of exposure was chosen to correspond to the duration of a complete spermatogenic cycle (54 days). There was no significant difference in final body weight between the two groups, but statistically significant decreases in left and right testicular mass were observed in the exposed group. The study showed no negative effect on rectal temperature and testicular histology. There were, instead, significant reductions in interstitial cell count /1000 µm2 (5.33 ± 0.56 to 4.47 ± 0.48; and borderline significant reduction of sperm motility and sperm distal cytoplasmic droplet. A significant increase in follicle-stimulating hormone levels was observed (13.44 ± 6.38 IU/ml to 26.96 ± 8.07 IU/ml. The authors also observed a slight increase in testosterone (1.44 ± 0.77 IU/ml; control vs. 2.30 ± 1.38 IU/ml; exposed group) and luteinizing hormone (9.17 ± 4.59 IU/ml; control vs. 12.58 ± 7.30 IU/ml; exposed group) concentrations in the exposed group compared with the control group.

The author discuss that the observed reduction in testicular weight could be caused by thermal effects due to oxidative stress or by IF magnetic field exposure. The heat generated by IF-MF exposure can increase body temperature and affect the testes. However, no significant changes in rectal temperature were observed. The significant increase in FSH may be due to changes in negative testicular feedback due to a decreased spermatogenic activity in the IF-MF group, which could lead to accelerated release of gonadotropin-releasing hormone (GnRH). Another possible reason includes increased gonadotropin production at the hypothalamic level due to IF-MF exposure.

3.3.2 Summary and conclusions on IF animal studies

With 150 kHz (0.3 V/cm) exposure, the study reports slight adverse effects on some fertility parameters and alteration of hormonal balance.

Table 3.3: Animal studies on exposure to IF fields

Endpoint	Reference	Exposure conditions	Species, strain, sex	Results
Fertility	Sundaram et al. (2022)	150 kHz, 12V 23 h/d, 7 d/wk, 8 wks (56 d)	Rat, Sprague-Dawley, male	Significant decreases in testicular mass, interstitial cell count/1000 μm2, sperm motility trajectories, and distal cytoplasmic droplets. Increase in FSH level and a slight increase in testosterone and luteinizing hormone concentrations.

Abbreviations: d: days; FSH: follicle-stimulating hormone; h: hour(s); IF: intermediate frequency; wk: week(s).

3.4 Cell studies

Only one study was identified published in 2022 on intermediate frequencies but it was not considered due to the low quality of the experimental procedure. It is reported in the table of excluded studies.

4. Radiofrequency (RF) fields

4.1 Epidemiological studies

Last years' report concluded that several studies were published on tumour risk in relation to mobile phone use that did not indicate increased risks of brain tumours with mobile phone use. A similar observation was reported from MobiKids, an international case-control study on mobile phone use and brain tumour risk in children, adolescents and young adults.
Last year's report also concluded that a finding on mobile phone use and fertility in normal-weight men (BMI < 25 kg/m^2) deserved follow-up investigation. For other outcomes, little progress was reported to understand presence or absence of effects on human health.

4.4.1 Child health

Bodewein et al. [49] systematically reviewed 42 epidemiological and 11 experimental studies (see also chapter 4.3) on RF-EMF exposure and child and adolescent health, in particular symptoms, cognition and behaviour. Study results were summarised taking the underlying quality of the studies into account. Overall, the authors judged the evidence of the epidemiological studies regarding associations between RF-EMF exposure and symptoms as well as cognitive function or behaviour change as low to inadequate. Evidence regarding infant development was judged to be inadequate. Human experimental studies were summarised with similar results, with inadequate evidence to draw conclusions regarding RF-EMF exposure and effects on cognition, brain activity and physiological changes in children and adolescents. In addition, as experimental studies only investigate acute and short-term effects, they do not allow conclusions regarding long-term effects. The authors reported that of 53 included studies, 35 had several methodological weaknesses affecting the internal validity of results. The authors state that it remained unclear whether children and adolescents were more vulnerable to RF-EMF exposure than adults. In addition, they stated that, while no clear evidence was found for adverse health effects, it could also not be concluded that RF-EMF exposure was safe for this age group, given that the evidence for this conclusion was too weak. A recommendation in light of rapidly developing technology was to perform high quality studies, such as cohort studies with improved exposure assessment, and experimental studies with larger study populations and different age groups.
This is a large and well-performed systematic review that highlights many of the shortcomings in the current literature, which reflects inherent challenges in RF-EMF exposure assessment, or the setting up of informative longitudinal studies in children, or performing large experimental studies. Of note, the few studies that managed to address RF-EMF exposure together with behavioural aspects of mobile device usage (e.g. behaviours related to evening and night-time use of mobile devices) indicate that behavioural aspects may be more relevant than RF-EMF exposure for adverse health effects. Because both are linked, effects are not always simple to disentangle, and this is rendered even more complicated by current technology use evolvement (more video less voice calling with a phone held to the ear). Given the pervasive use of the technology and the high prevalence of some of the outcomes, the topic remains of importance.

4.4.2 Adult cancer

Vijayan et al. [50] systematically reviewed and meta-analysed studies that assessed mobile phone use and salivary gland tumours. Seven case-control studies published between 2002 and 2012 were meta-analysed. No statistically increased risk among mobile phone users was identified, with a summary odds ratio of 1.06 (95%CI 0.86-1.32). No funnel plot asymmetry was observed, indicating no

systematic differences in risk estimates among studies with higher or lower precision (usually larger or smaller studies). The authors remark that given the study design, results were susceptible to selection and recall biases and that poor exposure assessment prevented firm conclusions.
As such, the meta-analysis is in line with one previous prospective (cohort) study on mobile phone subscribers (Schüz et al. 2006) that reported a standardised incidence ratio of 0.86 (0.56 to 1.26) in men (no cases in women were detected), and a letter published in 2009 (Shu et al Epidemiology 2009) exploring time trends of salivary gland tumours in Nordic countries and reporting that between 1970 and 2009, annual percent change was 0.1% (95% CI 0.4 to 0.2) for men and 0.2% (95%CI 0.5% to 0.1%) for women.

Deltour et al. [51] used the national cancer registry data from Denmark, Finland, Norway and Sweden to investigate how many brain tumour cases (gliomas) were diagnosed between 1979 and 2016, in men aged 40-69 years . This age group was primarily selected because they represent the main mobile phone user group in the Nordic Countries in the early stage when mobile phones were introduced. It was argued that any increased brain tumour risk should manifest itself first in this age group. Further, it was analysed to what extent observed glioma case numbers correspond to hypothetical risk scenarios as reported in previous epidemiological studies (Coureau et al. [52] , Hardell & Carlberg [53] , Momoli et al. [54] , Interphone Study Group [55]). Based on 18,232 glioma cases, glioma incidence increased slightly and steadily during 1979–2016 among 40–59-year-old and 60–69 -year-old men. It was demonstrated, however, that the observed time trends were not compatible with a risk from mobile phone use as reported in any of these four studies above. For men aged 40-59 years, relative risks of 1.08 or higher with 10 years of latency, a relative risk of 1.2 or higher with 15 years of latency, and a relative risk of 1.5 or higher with 20 years of latency are not compatible with the observed number of glioma cases in the Nordic Countries. For the age group 60-69 years, the same was true for relative risks of $\geq$1.4, $\geq$2 and $\geq$2.5 for latency periods of 10, 15 and 20 years, respectively. The authors conclude that increased risks of the magnitudes reported in some case-control studies are not plausible.
The study indicates that observed number of glioma cases in the Nordic countries are not compatible with a substantial risk from use of mobile phone. Although the study did not have individual exposure data available, a risk from mobile phone use would have to manifest itself in an increase in brain tumour incidence, as there are few other established environmental risk factors for brain tumours that have changed strongly over time and could compensate for any risk. A comprehensive report with data on other age groups and women was published in German by the Federal Office for Radiation Protection in Germany [56].

Schüz et al. [57] updated a prospective cohort study on mobile phone use and the risk of central nervous system (CNS) tumours (Benson [58]; summarized in SSM2014:16). The study included women from the Million Women Study, recruited between 1996 and 2001, who were mailed a new questionnaire around the years 2000-2003 (the "2001-questionniare"). After excluding 25,838 women with a previous history of cancer, incomplete questionnaire data, or neurofibromatosis (which predisposes for tumours), the final cohort consisted of 776,156 women aged 50-69. Of these women, 458,002 (with no previous cancer) filled out a new questionnaire around the years 2010-2012 ("2011-questionnaire"). The questionnaires inquired about mobile phone use, sociodemographics, lifestyle, and health. National public health registers provided data about all intracranial tumours, including glioma, glioblastoma, meningioma, acoustic neuroma, pituitary tumours, and other/unspecified intracranial tumours. The analyses were conducted both for the total number of cases and by laterality whenever possible. Additionally, the study examined the risk associated with tumours located in the temporal and parietal lobes, which are most exposed to radiation from a mobile phone held to the ear.

The main analysis used data from the 2001-questionnaire as the baseline and categorized participants as either never or ever users of a mobile phone. For mobile phone users, the analysis further grouped participants based on years of use (<5, 5-9, and 10+ years) and frequency of use (<once/day and daily use). In an analysis using the 2011-questionnaire as the baseline, the reference group was women who talked on their mobile phone for less than 1 minute per week. The exposed group was women who talked for 1 or more minutes per week, further subdivided by the minutes talked per week (1-<20 and 20+ min/week) and years of use (<10 and 10+). The study used Cox-proportional hazard models to analyse the data, accounting for age, year of birth, year of answering questionnaire, geographical region, area level deprivation, smoking, alcohol consumption, BMI, hormone therapy, and physical exercise. Comparing individuals who reported ever using a mobile phone to those who reported never using a mobile phone, the study found no statistically significant elevated risk for any tumour group in either questionnaire. In the 2001-questionnaire analysis, the observed hazard ratio (HR) for right-sided glioma was 1.17 (95% CI: 0.92-1.47), and for the glioblastoma subgroup, the HR was 1.23 (95%CI: 0.94-1.60). When subdividing users by frequency or duration of use, the highest point estimates were seen for those using their phone less than once per day and for less than 5 years. For acoustic neuroma, the HR for ever vs never use was 1.19 (95%CI: 0.89-1.59). Risk estimates were above one for those using the phone for 5 or more years but below one for those using the phone for fewer years, and there was no difference in risk estimates by frequency of use (less than 1 minute per day vs more). Regarding pituitary tumours, use of a mobile phone for less than 5 years was associated with the only statistically significant elevated risk (HR=2.16, 95%CI: 1.29-3.62). However, this finding was based on only 35 cases, and other exposure periods were associated with HRs below unity. The overall risk of pituitary tumours did not deviate from the null. The authors conclude "there was little to suggest that the use of cellular telephones increases the risk of brain tumours, overall or by subtype or by tumour location".
Some previous studies have suggested an association of heavy mobile phones with glioma or glioblastoma. Even though this study found somewhat elevated point estimates for these tumours, there was no statistical significance which indicates chance variation as the cause. Furthermore, the elevation was largely attributable to women with short or infrequent use, which speaks against a causal association and contrasts with previous studies were the association was seen for heavy use. Some studies have associated mobile phones with acoustic neuroma (AKA vestibular schwannoma). In this study high point estimates were seen for long term use (>5 years) but with no difference by frequency and never reaching statistical significance. This may result from chance or possibly detection bias where use of mobile phones increases likelihood of detecting hearing loss, a symptom of acoustic neuroma. Strengths of this study includes its large size and prospective nature. Limitations which may have reduced power to detect association include the relatively crude exposure assessment that does not allow for the rapid changes in phone usage, the time period of rapid uptake in mobile phone use around baseline, and also the relatively low use of mobile phones among women aged 50+ 20 years ago.

Elwood et al. [59] used national cancer register data to investigate incidence trends of all glioma, and of glioma situated in the temporal and frontal lobe (most exposed to a mobile phone held to the ear) in New Zealand in the years 1995 to 2020. They observed a total of 6677 incident glioma cases in people aged 10 or older. There was no indication of an increasing age-standardised incidence trend in people aged 10-69 or 20-39 for total glioma or for glioma of the temporal and parietal lobe. Among people aged 80 or older the incidence of glioma increased with an annual percentage change of 3.0 (95%CI: 1.0-4.9) for those age 80-85 and 5.4 (95%CI: 3.2-7.7) among those older than 85 years. Using mobile phone usage data from Australia, the authors calculated predicted incidence trends assuming that use of mobile phones was associated with a 30% increased risk for glioma and evaluated latency periods

of 5 to 20 years. Results showed that an association even assuming a long 20-year latency period should have been detectable in their data. The authors conclude that among New Zealanders aged 10-69, there was no increasing incidence of glioma in the period 1995 to 2020 The absence of a trend did therefore not support the hypothesis that mobile phone use increased the risk for developing glioma. Among the very old, the increasing incidence is consistent with improved diagnostics.
The lack of association reported here concords with previous studies. Incidence trend studies cannot rule out small effects or effects restricted to small user segments, but they are an important tool to evaluate the potential health impact of prevalent exposures such as mobile phones. The hypothetical mobile phone usage scenarios are by necessity limited by the available data on mobile phone usage in the population under study.

Peleg et al. [60] investigated a case series of 46 cancer patients who had reported exposure to RF-EMF during their military service as a possible cause for their cancer, and who had sought help from an NGO. This study was a follow-up to a previous case-series by Peleg et al. [61], which was summarized in SSM2019. Of the original 54 patients, 8 were excluded due to incomplete data on exposure or diagnosis. The study was conducted in 2019 and included patients diagnosed between 2002 and 2021, with ages ranging from 19 to 33 years at the time of diagnosis, and a latency from military recruitment ranging from 0.85 to 15 years. All cases reported working with or near military radar or radio equipment. The cancer diagnoses were reported by the patients and validated by medical records for 37 cases. The authors compared the percentage frequency (PF) constituted by specific tumour groups of the 46 cases to the percentage the same tumour groups constituted of all cancers in the Israeli cancer register. The observed PF for hematologic tumours was 41.3% (95%CI: 27-57) while the expected was 22.7%. For Hodgkin's lymphoma and sarcomas, the corresponding numbers were 21.7% (95%CI: 11-36) versus 11.6% and 15.2% (95%CI: 6.3-28.9) versus 7.0%, respectively. Six of the cases belonged to a group of 250 soldiers recruited together in 2011. Compared to data from the Israeli cancer register, the relative risk of cancer in this small group was 8.0 (95%CI: 2.9-17.0). The authors suggest that the observed risk estimates in this study may be attributed to RF radiation from radio and radar installations.
The study has major limitations. There was a lack of actual exposure assessment, and other potential exposures in these military functions were not considered. Additionally, the self-selection of patients who sought help from an NGO may not be representative of all cancer patients, which would bias the frequency analysis. The percentage frequency method applied has been shown to be problematic as it cannot be interpreted as an actual difference in risk between the comparison groups. It is unclear why the authors did not instead present metrics such as standardised incidence ratios. Also, the number of cases was small, increasing the risk for chance findings. Regarding the relative risk calculation in the 250-man unit, it should be noted that this group was identified because it had a high number of cancer cases. Therefore, the conclusion that this proved a generally increased risk of cancer in such units is not valid. The study does not provide data to suggest a similar incidence of cancer in other similar military groups nor does it provide data to indicate direct association with RF-radiation exposure.

4.4.3 Reproduction

To evaluate the association between electronic media use with semen volume, sperm concentration, total sperm count, total motility, progressive motility, and normal morphology, Chen et al. [62] evaluated 6608 semen donation records from 1454 healthy men, aged 22–45 years old, screened as potential sperm donors from April 2017 to July 2018 in Wuhan, China. Electronic devices use over the previous three months was collected via questionnaires by trained interviewers. Data was analysed with linear mixed-effect models adjusted for multiple confounders. Total duration of electronic device use was associated with reduced sperm progressive motility and reduced total motility. Duration of

mobile phone and computer use was associated with reduced sperm concentration, reduced progressive motility, and reduced total motility. Per daily hour increase of mobile phone call time sperm concentration decreased by 8.0% (95% CI: -15.2% to -0.2%) and total count by 12.7% (95% CI: -21.3% to - 3.1%). No associations for any sperm parameter were found with carrying mobile phones in trouser pockets or for the daily duration of having the mobile phone near the body (<50 cm). Stronger inverse associations were found for using a headset during a call, which reached statistical significance for total sperm count and total motility. No results on morphology were presented, possibly indicating that no associations were found for this parameter.
To date, this is the largest study on semen quality. A strength is the high number of confounders and the inclusion of healthy men screened as potential sperm donor. Previous studies have mostly included men that had consulted a clinic for fertility problems, which is likely to result in selection bias. Numerous analyses have been conducted without any correction for multiple testing. Thus, about 1-2 significant results would have been expected by chance. Overall, associations for mobile phone use are not notably stronger than for use of computers or all electronic device use combined. This suggests that observed associations may be rather caused by other aspects of electronic media use such as duration of sitting or lack of physical activity. These two factors have not been included in the analysis. Similar to all previous studies on this subject, a limitation is the RF-EMF exposure assessment, which is based on retrospectively self-reported data without considering emitting output power of the devices and the actual received dose.

Zhang et al. [63] conducted a cross-sectional study to investigate the effect of RF-EMF from mobile phones on semen parameters in 1634 men, who underwent semen examination at the Department of Reproductive Endocrinology, Zhejiang University, China between May 2020 and January 2021. A self-designed questionnaire was used to ask about mobile phone use and relevant sociodemographic variables and possible risk factors. In multiple linear regression models, adjusted for age and body mass index, daily duration of mobile phone use was negatively correlated with sperm motility but not with other sperm parameters such as sperm morphology, volume, concentration, DNA fragmentation index and total sperm number. Cumulative duration of mobile phone use, placement of the mobile phone on the body and whether earphones were used during mobile phone calls were not associated with any of the sperm parameters.
The large sample size is a strength of the study. Response rate was not reported. It is not described what the motivation of the study participants was to consult the hospital for a semen examination. Most likely, the participants had observed problems with their fertility, which makes the study population not representative for the general population. However, all semen parameters were within the normal range of WHO guidelines except the percentage of normal morphology, which was low. Given the high number of analyses that were conducted without multiple testing correction, one significant result is less than what would have been expected by chance. A limitation are the few confounders considered, although potentially more confounders might have been tested. Confounding from smoking and various chronic diseases was prevented by excluding such participants from the analysis. Overall, the study can be interpreted as indicating rather an absence of RF-EMF effects on sperm quality.

4.4.4 Self-reported electromagnetic hypersensitivity (EHS) and symptoms

Farashi et al. [64] conducted a systematic review on the effects of EMF exposure from mobile phones on headaches. Based on 30 eligible studies a pooled odds ratio of 1.30 (95% CI 1.21–1.39) was computed with significant heterogeneity between studies.
The quality of this meta-analysis is low as expressed in a letter to the editor by Jalilian et al (2022) [65]. Main criticisms include the mixing of human experimental and epidemiological studies to one

single effect estimate, no attempt to quantify intensity of exposure or duration of mobile phone use, omission of eligible papers, intransparent risk of bias, inaccurate data extraction and inclusion of multiple estimates per study in the meta-analysis, which results in an underestimation of the confidence intervals.

A study by Traini et al. [66] investigated which factors play a role in attributing individually existing health complaints to electromagnetic hypersensitivity (EHS) with regard to RF-EMF, and how this attribution possibly changes over time. For this purpose, 892 participants of a cohort study in the Netherlands were interviewed at three time points, first in 2011/2012, then again in 2013, and finally in 2021. Self-reported data on RF-EMF exposure, RF-EMF risk, non-specific symptoms, sleep problems, and self-declared EHS were collected. The mean age at the beginning of the study was 50 years (52% women). At each of the three survey time points, about 1% of the participants attributed health complaints to RF-EMF exposure. While the prevalence remained the same over the study period, the individual attribution of symptoms to EMF was much more dynamic over time. After 10 years, people attributing symptoms to EMF at baseline had a 95% chance of transitioning to no attribution. In the last survey in 2021, the study participants were asked how electrosensitive they considered themselves to be on a scale of 1-6. Out of 892 respondents, 12.1% gave a score between 4-6 and were classified as EHS. Participants who perceived their EMF exposure and the associated risk as high were more likely to declare themselves as EHS. According to the authors, a better understanding of the influencing factors and dynamics in the attribution of symptoms to EMF is helpful for future risk communication.
The survey shows that the way of asking about EHS has a big influence on the reported prevalence of symptom attribution or EHS. Strengths of the study include the long follow-up time with acceptable response rate. A weakness of the study is the small number of people at the start of the study (9 people) who attributed symptoms to RF-EMF. Random errors could therefore have influenced the results. Nevertheless, the study is in line with other research. It seems that EHS is not a very stable attribution and often changes over time, even if the overall proportion in the population may remain constant.

4.4.5 Other outcomes

El Jarrah et al. [67] present a systematic review of exposure to mobile phone use during pregnancy and child health. However, the applied methods appear problematic: They retrieved 18 papers published in the past five years, of which only nine actually report on child health. Of these nine studies, one was a modelling study to assess exposure (and not health), one study was an experimental study in rats, and one evaluated effects of extremely low-frequency field exposure. Such studies cannot be combined to an overall assessment of the evidence. In addition, other studies that were published in the respective time frame and that appear eligible according to the inclusion criteria were missing, such as Sudan et al 2017, or Papadopoulou et al, 2017. Overall, this systematic review does not contribute to the question if maternal radiofrequency electromagnetic field exposure during pregnancy has an effect on health of their offspring.

Girela-Serrano et al. [68] systematically reviewed publications on mobile phone use or wireless device use and mental health in children and adolescents. Overall, 25 studies were identified, which included 15 cohort and 15 cross-sectional analyses. The authors reported “suggestive but limited evidence” that mobile phone use or wireless device use was associated with poorer mental health in children or adolescents. They also reported substantial heterogeneity in design, measurement of mobile phone or wireless device use and mental health outcomes. Only one reviewed study attempted to disentangle behavioural aspects from radiofrequency electromagnetic field exposure, and that study did not indicate that RF-EMF was associated with mental health of the studied adolescents.

Amiri et al. [69] used data from the Iranian Ravansar non-communicable diseases (RaNCD) study of adults aged between 35 and 65 years to conduct a cross-sectional analysis on the association of self-reported duration of mobile phone use with blood pressure and heart rate. The study started in 2014 but the date of the cross-sectional survey was not specified in the paper. Complete data for this analysis was available from 8905 out of 10,065 RaNCD study participants. Data was analysed by multiple linear regression adjusted for age gender, smoking status, residential areas, education level, socioeconomic status, BMI, physical activity, kidney disease, depression, psychiatric disorder and cardiovascular disease. In total, 1515 (17.0%) of the study participants did not use mobile phones. The remaining participants were classified into four quartiles according to their daily mobile phone use in the twelve previous months: Q1: 3.4 min/day; Q2: 8 min/day; Q3: 16.2 min/day; Q4: 50.4 min/day. Mean systolic blood pressure was 107.9 mmHg in men and 104.2 mmHg in women. Diastolic blood pressure was 70.0 mmHg in men and 67.8 mmHg in women. Mean heart rate was 72.2 beats per minute in men and 75.2 in women. Mobile phone use was associated with a decrease in systolic and diastolic blood pressure in an exposure-response manner but no overall association with heart rate was observed. Apparently, associations were restricted to women, but no confounding adjusted, or gender-stratified results were presented.
The large population-based sample size is an asset of this study. A limitation is the cross-sectional design and retrospectively self-reported mobile phone use data without considering any information about the output power of the phones or received dose of the heart or brain. Various confounding factors were considered, and results were relatively robust in terms of various choices for confounding adjustment. Nevertheless, it cannot be ruled out that observed associations are due to residual confounding, including measurement errors in the included confounders, or missing lifestyle factors such as nutritional factors or environmental exposures (e.g., noise).

Cao et al. [70] published a paper including meta-analysis of mobile phone use in relation to endpoints ranging from accidents and neoplasms to ADHD and wrist injuries. The literature search is very inadequate. E.g., for cancer only studies by Luo and Hardell were included, and several results were based only on multiple reports from a single publication. The study is uninformative.

4.4.6 Conclusions on epidemiological studies

In the last year, most research addressed cancer risks in adults from using mobile phones. Two incidence trend studies did not find indications that brain tumour incidence has increased because of mobile phone use. These studies demonstrate that risk increase in the range of 20% or higher after 15 years can be excluded. A large prospective cohort study of women did not find an association between mobile phone use and brain tumours.
Two recent large studies have reported a few associations between different aspects of mobile phone use and some semen quality parameters, although the majority of analyses did not indicate increased risks. Given the high number of analyses, this may represent chance findings but needs follow-up investigations.
A prospective cohort study from the Netherlands indicates that EHS is not a very stable attribution and often changes over time, even if the overall proportion in the population may remain constant.
An Iranian study found a decrease of blood pressure in relation to mobile phone use in women but not men. Given the lack of mechanism and lack of supporting data from other studies, this may be a chance finding or due to residual confounding.

Table 4.1: RF-EMF exposure and health, epidemiological studies

Endpoints	Reference	Exposure assessment	Study design, population	Results
Glioma (meningioma)	Deltour et al, 2022	Population aggregated subscriber rates	Incidence trends study: All adults from Denmark, Finland, Norway and Sweden	No changes in glioma incidence in the Nordic countries have occurred that are consistent with a substantial risk attributable to mobile phone use.
Glioma, incidence trends	Elwood et al, 2022	Glioma incidence: NZ-cancer register. Mobile phone usage data: Australian administrative data	New Zealand Incidence trend study, age 10+, years 1995-2022. 6677 incident gliomas.	Incidence of glioma, overall or in the most exposed parts of the brain did not rise in a way reflecting the increasing use of mobile phones.
CNS tumours	Schüz et al, 2022	Self-reported duration and frequency of mobile phone use at baseline	Cohort study: 776,156 women aged 50-69 from a UK cohort with questionnaires around 2001 and 2011	Mobile phone use not associated with CNS tumours.
Salivary gland tumours	Viayan et al, 2022	Not applicable	Systematic review, seven case-control studies published 2002-2012	No statistically significant increased risk among mobile phone users .
Cancer	Peleg et al, 2022	Self-reported military occupation involving radio or radar equipment	Case series, 46 Israeli cancer cases aged 19-33 and diagnosed between 2002 and 2021.	A tendency for different tumour distribution among identified cases than among cases in the Israeli cancer register. Many methodological weaknesses.
Blood pressure and heart rate	Amiri et al, 2022	self-reported duration of mobile phone use	Cross-sectional: 8905 adults aged between 35 and 65 years from a cohort study in Iran	Mobile phone use was associated with a decrease in systolic and diastolic blood pressure in women but not men. No association with heart rate was observed. Unclear mechanism, cross-sectional study design a weakness..
Semen quality (volume, concentration, count, motility, morphology)	Chen et al, 2022	Self reported electronic devices use over the previous three months.	Cross-sectional: 1454 healthy men, aged 22–45 years old, screened as potential sperm donors from April 2017 to July 2018 in Wuhan, China	Majority of exposure-outcome combinations were not significant, but some inverse associations observed. Overall, the result pattern does not follow an expected dose-response pattern.
Semen quality (motility, morphology, volume, concentration, DNA fragmentation index and number)	Zhang et al, 2022	Self-reported mobile phone use	Cross-sectional: 1634 men, who underwent semen examination at the Department of Reproductive Endocrinology, Zhejiang University, China between May 2020 and January 2021.	Daily duration of mobile phone use was negatively correlated with sperm motility and not associated with other sperm parameters such as sperm morphology, volume, concentration, DNA fragmentation index and total sperm number.
Pregnancy outcomes	El Jarrah et al, 2022	Not applicable	Systematic review	Not informative review
Symptoms, cognition, behaviour in children	Bodewein et al, 2022	Not applicable	Systematic review; 42 epidemiological studies included	Unclear if children are more vulnerable to RF-EMF exposure. No clear evidence of effects on children but due to low quality of many of the underlying studies, it also cannot be concluded that RF-EMF exposure is safe for this age group.
Childhood cognition	Girela-Serrano et al, 2022	Not applicable	Systematic review of 25 studies, with 15 cohort and 15 cross-sectional analyses performed in children and adolescents.	Authors report "Suggestive but limited evidence of wireless device use with poorer mental health", indicating primarily behavioural aspects of device use to be associated with mental health.
Factors related to EHS	Traini et al, 2022	Not relevant	Cohort study: 892 adults (mean age 50 at baseline, 52%women) from a Dutch cohort recruited it 2011/2012	After 10 years, people attributing symptoms to EMF at baseline had a 95% chance of transitioning to no attribution. Participants who perceived their EMF exposure and the associated risk as high were

				more likely to declare themselves as EHS.
"All" endpoints	Cao et al, 2022	Meta-analysis	Meta-analysis	Incomplete literature search, not informative
Headache	Farashi et al, 2022	Meta-analysis	Systematic review	Incomplete literature, faulty methodology, not informative study.

4.2 Human studies

While the original studies published in 2021 investigated RF-EMF effects on brain activity, those published in 2022 investigated effects of RF-EMF exposure on physiological outcome measures (blood pressure (BP), heart rate (HR) and heart rate variability (HRV), Huang et al. [71], on the functional connectivity between intrinsic connectivity networks (Yang et al. [72]), and food intake (Wardzinski et al. [73]).

In 2022 two systematic literature reviews and three original research papers investigating RF-EMF effects in human experimental studies were published. The systematic reviews addressed effects from wireless communication devices on children and adolescents (Bodewein et al. [49]) and effects on the brain (Hinrikus et al. [74]).

The study by Bodewein et al. [49], which reviewed epidemiological and experimental studies has been discussed in more detail in chapter 4.1. Out of the 11 experimental studies nine investigated RF-EMF exposure effects on brain activity (EEG-based: nine studies) and cognitive function (four studies), another two investigated different physiological parameters (e.g. heart rate and respiratory rate). The authors concluded that evidence from the experimental studies is inadequate to draw a conclusion regarding mobile phone-related exposure and its effects on cognition, brain activity, and physiological changes in children.

In order to investigate differences in RF-EMF effects between various generations of telecommunication systems and to project possible effects on the 5G technology, Hinrikus et al. [74] performed a review on possible health effect on the human brain. The authors searched the EMF-Portal database for publications with the filters "experimental studies", "radiofrequency (≥ 10 MHz)" and "mobile communication". In a second step the search words EEG or ERP or cognition of behaviour were applied. After excluding animal studies, five quality criteria were applied: 1) a minimal sample size of 10, 2) inclusion of at least one sham or control condition, 3) an at least single-blind design, 4) correctly determined exposure conditions, i.e. measured field strength or power density, and 5) correct statistical evaluation. Applying these criteria 73 studies were included in the review. The results indicated no consistent relationship between outcome measures (sleep EEG, sleep quality, event related potentials, cognition, behaviour and brain metabolism) and parameters of exposure from different generations (2G, 3G, and 4G) of telecommunication technology. Based on these results the authors hypothesize that the impact of exposure from 5G in the NR FR1 frequency range (up to 10 GHz) is principally not different from the one of previous generations of mobile communication. However, the authors claim that since mechanisms underlying possible RF-EMF effects are not yet known, and since there is lack of experimental in vivo studies of 5G FR2 exposure further studies are needed.

Huang et al. [71] exposed 58 individuals with self-reported idiopathic environmental intolerance attributed to EMF (IEI-EMF) and 92 individuals without IEI-EMF as control group (age range 20 to 69 years) to EMF signals mimicking those from mobile phone base stations. A spherical near-field measurement system antenna was used to generate signals mimicking EMF from 2G 900 MHz GSM and 1800 MHz GSM as well as from 3G base stations of GSM 800 MHz and GSM 2100 MHz. The peak power was set at 0.25 W/m^2 for an average combined power of 1 W/m^2. Both exposure conditions, real and sham (duration 30 min each) were applied in a double-blind randomized cross-over design within groups in one test session (duration 120 min) with a 30 min washout period between exposures. After 15 min of exposure and at the end, the subjects recorded whether they perceived exposure and whether they experienced any symptoms. The physiological measurements were recorded throughout all experimental session at 5-min time intervals. The samples differed significantly with regard to age and sex distribution, occupation, and self-perceived health status. IEI subjects reported more symptoms than the control subjects during both the real and the sham exposure session. However, none of the symptoms was specifically related to EMF exposure. The physiological parameters systolic blood pressure (BP), diastolic BP, mean arterial BP, heart rate (HR), the low and the high frequency component of heart rate variability (HRV) and their ratio, however, were not significantly different between groups in the sham condition. The results indicated that the participants of both groups were not able to accurately perceive the EMF exposure status. In the IEI-EMF group 75.9% of participants reported having symptoms during the provocation session, while 81.0% experienced symptoms during the sham session. Overall, the participants with IEI-EMF experiences more symptoms than the control group (provocation session: 25.7%; sham session: 19.6%). The physiological parameters did not differ significantly between exposure sessions, neither in the IEI-EMF nor in the control group. The results revealed that except for the LF/HF ratio in the IEI-EMF group, the differences in physiological parameters were generally larger between sessions with and without perceived EMF exposure than between sessions with and without actual exposure. This study adds evidence, that symptoms including physiological parameters are not causally linked to short-term RF-EMF exposure in subjects with self-reported idiopathic environmental intolerance attributed to EMF (IEI-EMF).

Yang et al. [72] pursued to investigate effects of head exposure to an LTE signal on functional brain networks (Yang et al. [72], SSM last year's report [1]). The study was performed with 17 healthy young participants (9 men and 8 women, age range 18-38 years, mean ± SD: 26.1 ± 4.2 years) in a double-blind randomized counterbalanced cross-over design. Sessions were scheduled with a time interval of one week. Prior to and following a 30 min LTE or sham exposure functional connectivity was measured with functional magnetic resonance imaging (fMRI) with a 3.0T scanner. Structural MRI was conducted before the two sessions. LTE exposure at 2.573 GHz was delivered by a signal generator. The calculated peak SAR_{10g} value was below 2 W/kg for all participants during real exposure, with a mean ± SD of 1.22 ± 0.24 W/kg. Group-level independent component analysis was applied to decompose networks of interest. Fourteen intrinsic connectivity networks (ICNs) were identified. Dynamic connectivity as well as conventional connectivity between networks per state were computed and followed by paired sample *t*-tests. Results showed that there was no statistically significant difference in terms of static and dynamic functional networks connectivity both in the sham and the LTE exposure condition. The authors concluded that the short term exposure was insufficient to be detected at the ICN level. As for the previous study a limitation is that the vigilance level of the subjects while they were in the scanner was not controlled. Although participants reported that they kept conscious during the experiments it cannot completely be ruled out that results are affected by this factor. Furthermore, the method of assessment of networks, fMRI, includes exposure to various EMF fields, among them RF-fields, which might have masked the additional experimental LTE exposure effect.

Given the simultaneous spread of obesity and mobile phone use and that RF-EMFs emitted from mobile phones are largely absorbed by the head, Wardzinski et al. [73] investigated whether RF-EMF exposure might affect food intake. Fifteen normal-weight young men (age range 20-29 years; mean ± SEM: 23.47 ± 0.68 years; BMI: range 21.5 – 24.5 kg/m^2) participated in this single-blind, sham controlled, randomized cross-over study. Subjects with a medical condition were excluded and were only enrolled if they had a regular sleep-wake cycle four weeks prior to testing and did not participate in shift work.

Participants were instructed to abstain from food and caffeine for 12 h before the experiments and no mobile phone use was allowed for 12h prior to the experiments. Exposure was delivered for 25 min by two different types of commercially available mobile phones (Motorola L2, SAR: 0.97 W/kg and Nokia 5800d-1, SAR: 1.33 W/kg), both transmitting in the GSM 900 MHz standard. An unvarying reception quality was ensured by continuous transmission via a base station simulator. Mobile phones were installed in a compact headset so that the phones were not visible for the participants. For the sham condition a deactivated phone was used. The three sessions were scheduled with a two week interval between assessments. Procedures started at 06:30 in the morning. To ensure that all participants were fasting at the start of the measurements, blood glucose levels were determined. Blood for the assessment of glucose, insulin and C-peptide levels, which are known to influence glucose metabolism and appetite regulation was sampled by a cannula inserted into an antecubital vein. Prior to any exposure a baseline ^{31}phosphorus magnetic resonance spectroscopy (^{31}P-MRS) sequence was recorded. After 5 min of exposure to RF or sham second ^{31}P-MRS was recorded in order to investigate possible immediate effects on the brain metabolism. Thereafter subjects were exposed for another 20 min, followed by the recording of a series of 5 continuous ^{31}P-MRS sequences. At 08:30 a.m. a standardized breakfast buffet was offered. For 40 min participants were allowed to eat ad libitum. Subjects were not aware that food intake was a measure of interest in this experiment, nor did they know that their individual food consumption was quantified by weighing buffet components before and after the meal. Blood samples were obtained at baseline, at the end of the first mobile phone/sham phone exposure, at the end of the following spectroscopy recording, at the beginning and the end of the 20 min of mobile phone/sham phone exposure, at 10 min intervals during the 5 continuous spectroscopy sequences, and before and after the buffet test. The results indicate a significant increase in total calorie consumption after both RF exposure conditions as compared to sham (22% for the Motorola phone and 27% for the Nokia phone). The increase was observed in 13 of the 15 subjects. The respective difference in calorie consumption between phones was not significant. With regard to macronutrients, the differences are specifically reflected in the intake of carbohydrates, protein intake was also enhanced. Fat intake reached statistical significance only for the phone with the higher SAR value. Again, differences between phones were negligible. The concentrations of blood glucose, insulin, and C-peptide, which were monitored throughout the study since appetite regulation is intrinsically related to glucose metabolism, were not affected by RF exposure at all times of assessment. Results from ^{31}P-MRS assessments indicate an increase in the adenosine triphosphate and phosphocreatine ratios to inorganic phosphate after RF exposure from mobile phones, reflecting a change in the cerebral high-energy phosphate metabolism, which is closely related to food intake and body weight. The authors emphasize that the observed RF-EMF induced alterations of the brain homeostasis might be of broader interest since a balanced central nervous energy homeostasis is of fundamental interest for all brain functions. This study was commented by Witthöft et al. [75]. They criticize the small sample size, an unbalanced experimental within-subject design with two RF exposures and one sham exposure, which might have introduced uncontrolled order effects, and a lack of a blinding check (e.g. unblinding could have occurred due to heating in the RF exposure condition). Finally, they question that exposure impacting mainly the right temporal region can increase the metabolism of another region leading to such an increase in calorie consumption. For a reply to these comments see Wardzinski et al. [76] . Independent replication studies, which are certainly necessary to confirm or disprove the present results, should be performed as double-blind-studies. Sample size for a given power and error probability should be based on the present findings. If more than one real RF exposure condition is involved, order should be considered as a factor in the statistical analysis, and – where applicable – corrections for multiple testing should be considered. Finally, a detailed dosimetric assessment of RF exposure including measurements is necessary.

4.2.1 Conclusion human studies:

One systematic literature review concludes that evidence from human experimental studies is inadequate to draw conclusions on mobile phone-related exposure effects on brain activity including cognition (Bodewein et al. 2022). Another indicates that is no consistent relationship between outcome measures (sleep EEG, sleep quality, event related potentials, cognition, behaviour and brain metabolism) and parameters of exposure from different generations (2G, 3G, and 4G) of telecommunication technology (Hinrikus et al. 2022). Based on these results it is hypothesized that the impact of exposure from 5G in the NR FR1 frequency range (up to 10 GHz) is principally not different from the one of previous generations of mobile communication.

One of the three original research papers (Yang et al. 2022) pursued to investigate LTE exposure effects on functional connectivity and brain network properties. Results showed that there was no statistically significant difference in terms of static and dynamic functional networks connectivity both in the sham and the LTE exposure condition, which is in line with previous results (SSM last year's report). The results of another study adds evidence, that symptoms including physiological parameters are not causally linked to RF-EMF exposure in subjects with self-reported idiopathic environmental intolerance attributed to EMF (IEI-EMF).

Finally, a study by Wardzinski et al. (2022) observed a relation between mobile phone exposure and food ingestion and brain energy homeostasis. The study, which is criticized for several reasons, certainly needs independent replication studies, which are certainly necessary to confirm or disprove the present results, should be performed as double-blind-studies. Sample size for a given power and error probability should be based on the present findings. If more than one real RF exposure condition is involved, order should be considered as a factor in the statistical analysis, and – where applicable – corrections for multiple testing should be considered. Finally, a detailed dosimetric assessment of RF exposure including measurements is necessary.

Table 4.2: Human studies on exposure to RF fields

Endpoints	Reference	Exposure condition	Sample	Results
Symptoms and physiological parameters	Huang et al. (2022)	30 min exposure (real or sham) to EMF signals mimicking those from mobile phone base: 2G 900 MHz GSM and 1800 MHz GSM as well as from 3G base stations of 800 MHz GSM and GSM 2100 MHz. The peak power was set at 0.25 W/m^2 for an average combined power of 1 W/m^2.	58 subjects with IEI-EMF and 92 controls (age range 20 to 69 years)	Number of reported symptoms were similar in both groups during both the provocation and sham exposure. No participant could accurately identify the exposure situation. Both groups: no significant differences in physiological parameters: systolic and diastolic BL, heart rate, low and high frequency component of heart rate variability and their ratio between sham and real exposure.
Functional brain networks	Yang et al. (2022)	30 min exposure (real or sham); real exposure: LTE exposure at 2.573 GHz delivered by a signal generator. Calculated peak SAR_{10g} value < 2 W/kg for all participants during real	17 healthy young participants (9 men and 8 women, age range 18-38 years, mean ±	No statistical difference in static and dynamic functional network connectivity in both real and sham exposure conditions.

		exposure, mean ± SD of 1.22 ± 0.24 W/kg.	SD: 26.1 ± 4.2 years)	
Food intake, blood levels of glucose, insulin and C-peptide as well as cerebral high-energy phosphate metabolism	Wardzinski et al. (2022)	25 min exposure to RF emitted from two mobile phones with different SAR levels: Motorola L2: 0.97 W/kg and Nokia 5800d-1, 1.33W/kg	15 healthy normal-weight young men (age range 20-29 years; mean ± SEM: 23.47 ± 0.68 years)	Increased calorie consumption after RF exposure as compared to sham: Motorola L2: 22%, Nokia 5800d-1: 27%; at no time point of assessment differences in blood levels of glucose, insulin, and C-peptide; increase in the adenosine triphosphate and phosphocreatine ratios to inorganic phosphate after RF exposure from mobile phones, reflecting a change in the cerebral high-energy phosphate metabolism

4.3 Animal studies

Last year, most included studies showed some effects of exposure, a few did not. The exposure parameters, such as frequency, duration and exposure level, varied considerably between studies. Therefore, no general conclusions could be drawn, 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 continued to be found, in contrast to effects on memory and behaviour.

4.3.1 Effects on brain

Souffi et al. [77] exposed male Wistar rats to the head only with 1800 MHz LTE fields for 2 h. The SAR in the region of interest, the auditory cortex, was 0.5 W/kg. Rats were either subjected to acute neuroinflammation induced by lipopolysaccharide (n=11 per group), or they were healthy (n=5 per group). In the diseased animals, exposure (compared to sham exposure) resulted in a reduction in the response strength to pure tones and to natural vocalizations, together with an increase in acoustic threshold in the low and medium frequencies. Immunohistochemistry showed no change in the area covered by microglia cell bodies and processes. No effects were observed in the healthy animals.

Orlacchio et al. [78] exposed female C57Bl6 mice under anaesthesia locally to the brain using 1.8 GHz for 210 seconds at a brain-averaged SAR of 2 or 6 W/kg (n=5 or 6 per group). They then used a neuroimaging technique based on a functional ultrasound (fUS) probe to observe the areas of brain activation during the RF-EMF exposure with high spatial and temporal resolution (~100 µm, 1 ms) following manual whisker stimulation using a brush. No changes were observed in the time course of the evoked fUS response in the left barrel field cortex.

Liu et al. [79] exposed male Sprague Dawley rats (n=20 per group) to 9.37 GHz EMF, pulsed at 1.875 Hz, for 2 min at a mean whole-body SAR of 6.08 W/kg. Spatial learning and memory were impaired in the exposed group. This effect was attenuated by administration of 20-hydroxyecdysone, which facilitated neurogenesis in the subgranular zone of the brain.

Dasdag et al. [80] exposed male Wistar rats (n=8 per group) to a 2.4 GHz WiFi signal, 24 h per day for 1 year. The whole-body SAR was 0.000328 W/kg and the brain SAR was 0.000655 W/kg. At the end of the study, the rats were sacrificed, and brains were removed to analyse expression of 10 specific microRNAs and membrane and depot fatty acids of brain cells. The expression of the microRNA rno-miR-181a-5p and of the fatty acids phosphatidylserine and triacylglycerol were increased in the exposed group. The possible health implications of this are unknown. No effects were found on other microRNAs and fatty acids.

Tümkaya et al. [81] continuously exposed pregnant Sprague Dawley rats (n=5 per group) to 900 MHz EMF until the end of pregnancy at a whole-body SAR of 0.087 W/kg. In the offspring, auditory brainstem responses were recorded on postnatal day 13, when hearing starts (number of animals not provided). No significant difference was observed between the average latency of waves in the exposed and sham-exposed groups. New-born rats were sacrificed on days 7, 10, 15, and 30 (n=6 per group). In the exposed animals, structural damage in cochlear nuclear neurons and oligodendrocyte cell structures and increased caspase-3 activity were observed, but there were no differences between the observation times.

Tan et al. [82] exposed Wistar rats to 2450 MHz EMF for 12 h/day at a brain SAR of 0.071 W/kg (whole-body SAR 0.093 W/kg). In the first generation the groups consisted of 1 male and 3 females. In the first group, only the male was exposed, in the second group only the females and in the third groups both male and females were exposed. After 1 month exposure, the females were fertilized. One female was sacrificed after 18 days and the foetuses removed for investigation, the other two females completed the pregnancy. When the offspring was 2 months old, the procedure was repeated, and this was again repeated twice until the 4th generation. In all groups of all generations, development of the brain in foetuses, histopathological changes in the brain of female rats and MAPK proteins in the hippocampus of male rats were investigated (n=4-14 per group). In all generations, haemorrhagic areas, irregular cellular localization and vascular structures were found in the brain of foetal and adult female rats and increased levels of pERK, ptau, pJNK and pP38 in the brain of adult male rats. Differences between the 3 exposure groups (male, female, both) were observed with some endpoints, but there was no clear pattern.

Bektas et al. [83] exposed male Wistar rats (n=7 per group) to 3.5 GHz EMF, 2 h per day for 30 days. The brain SAR was 0.00195 W/kg. Half of the animals was made diabetic by administration of streptozotocin. In all exposed groups – compared to sham-exposed animals, the oxidative stress in the brain was increased (total antioxidant was decreased, total oxidant and hydrogen peroxide were increased). In addition, the levels of two hormones that influence food intake were changed: ghrelin was increased and nesfatin-1 was decreased in the brain. The hormone irisin, thought to have potential in the treatment of metabolic diseases, especially obesity and diabetes, was increased in the brain. Also, the number of degenerated neurons in the hippocampus was increased.
The statistical analysis is not always clear, the number of degenerated neurons was not numerically analysed.

Kopani et al. [84] exposed New Zealand White rabbits (n=5 per group; sex not given) to 1805-1870 MHz EMF for 150 min at an E-field strength of 300 V/m and/or 1788 MHz EMF, pulse modulated 50%, for 150 min at an E-field strength of 160 V/m. They then measured iron concentration in the cerebellum as an indicator of disruption of the blood-brain barrier. No differences between the exposed and sham-exposed groups were observed.

4.3.2 Cognition, behaviour

Perov et al. [18] exposed male Wistar rats (n=12 per group) to combined 5G frequencies of 3.6, 28 and 37 GHz, continuously for 4 months. The power density was 250 μW/cm^2. The exploratory activity using the open field test and blood concentrations of ACTH and corticosterone were evaluated at the end of each month of exposure and 1 month after exposure. Only after 2 months of exposure a significant decrease in exploratory activity was found, at 1 month after cessation of the exposure the level was back to normal. There was no effect on the ACTH level, while corticosterone was increased at 1 and 2 months during exposure and at 1 month after cessation of the exposure, but not at 3 and 4 months during exposure.

Li et al. [85] exposed male Wistar rats (n=14-17 per group) to 2.856 GHz EMF for 6 min three times a week for up to 6 weeks. The average power density was 30 mW/cm^2. They investigated the effect of exposure on 5-hydroxytryptamine (5-HT), that plays an important role in cognition, emotion and brain development. The 5-HT1A receptor plays a regulatory role in the 5-HT system as one of the main mediators of the action of 5-HT and a potential target for enhancing cognition. The rats were grouped into 3 genotype groups of the 5-HT1A receptor: rs198585630 TT, rs198585630 TC and rs198585630 CC groups, in order to investigate whether these genotypes affected the cognitive ability and the response to RF EMF exposure. They observed that rats carrying the rs198585630 C allele exhibited increased mRNA and protein expression of the 5-HT1A receptor in the hippocampus and were more susceptible to RF EMF exposure, showing cognitive deficits and inhibition of brain electrical activity. The brain SAR was stated to be as high as 17 W/kg, but no method for this assessment was provided. At this high level of exposure, thermal effects are possible.

Dasgupta et al. [86] exposed zebrafish embryos to 3.5 GHz EMF from 6 to 48 h after fertilization at a whole-body SAR of 8.27 W/kg. When the fish reached adulthood, they assessed behavioural aspects. The avoidance response to predators was increased and the schooling response to other zebrafish was decreased in the animals exposed in the embryonic stage. Using mRNA sequencing, they also observed a modest transcriptomic disruption in the embryos directly after the exposure, with 28 differentially expressed genes. In addition, biochemical pathways related to metabolism were significantly perturbed.
The reported SAR was stated to be estimated, but no method was provided. The number of animals per group in the behavioural studies is not provided but is likely high (as they looked at schooling).

Vargová et al. [87] exposed female *Ixodes ricinus* ticks to 900 MHz EMF for 25 h, either in a tube or in a circular open-field arena that were both partly shielded and placed in an anechoic chamber. The exposure level in the tube was 1 mW/m^2 (0.6 V/m) and in the open field 0.61 V/m in the exposed part and 0.09 V/m in the shielded part. In the tube, 140 animals per group were tested. There was no difference in preference for the irradiated and shielded parts of the tube. In the open-field, 160 animals were used. The time spent and the trajectory passed was significantly longer in the part of the arena exposed to EMF.

Hao et al. [88] exposed C57BL/6N mice to 300 kV/m electromagnetic pulses with a centre frequency of 100 MHz, 400 pulses at a frequency of 1 Hz. They observed a decrease in spatial learning and memory, but no effects on avoidance learning and memory, novelty-seeking behaviour, and anxiety (n=10-12 mice per group). Neuronal activity in the hippocampus (measured in real time using a fibre optics technique in 3 mice) was inhibited during exposure but recovered immediately after exposure. At 1 day after exposure during memory and learning tests, neuronal activity was decreased, while at 7 days after exposure some recovery occurred. They also observed damaged structures in hippocampal neurons after exposure.

Aeen et al. [89] exposed male Sprague Dawley rats (n=8 per group) to 2.45 GHz, 500 Hz pulse modulated, EMF for 45 min per day during 4 or 8 days. The power density was 4 mW/cm^2. Spatial memory measured by the Morris water maze 24 h after exposure was decreased and anxiety, measured by the open field test, was increased. In the group exposed for 8 days, serum globulin was decreased, while no effect was found on albumin and total serum protein. Also, the reaction time in the hot plate test was increased. No such effects were found in the group exposed for 4 days.

Wang et al. [90] exposed male Wistar rats (n=30 per group) for 6 min to either 1.5 GHz at a whole-body SAR of 3.3 W/kg, 4.3 GHz at a whole-body SAR of 2.5 W/kg, or both frequencies simultaneously. Spatial learning and memory, cortical electrical activity and hippocampal ultrastructure were assessed by the Morris water maze, electroencephalography, and transmission electron microscopy, respectively. Impaired spatial learning and memory, decreased cortical excitability and damage to the hippocampal ultrastructure were observed in the exposed groups, especially the ones exposed to 1.5 GHz and at both frequencies. A total of 54, 145 and 296 exosomal proteins were differentially expressed between the sham exposed group and 1.5 GHz, 4.3 GHz and combined exposure groups, respectively. These proteins were involved in the synaptic vesicle cycle and vesicular transport.

In a second paper of this group, Wang et al. [91] exposed male Wistar rats (n=30 per group) for 6 min to 1.5 GHz to the whole body at 3 exposure levels: 5, 30 and 50 mW/cm^2 (50, 300 and 500 W/m^2). They calculated brain SARs of 1.85, 11.1 or 18.5 W/kg, respectively. Rectal and surface temperature measurements showed a maximum core temperature increase of approximately 1 °C. Rats exposed to the two highest SAR levels showed exposure level-dependent impaired spatial memory, inhibited EEG activity, pyknosis and hyperchromatism of neuron nucleus and changes in NMDAR subunits and downstream signalling molecules.
They did not measure brain temperature, however; the highest exposure level of 500 W/m^2 is possible to result in thermal effects, which might be counteracted by changes in metabolism by the animals. These might explain – part – of the observed effects.

Yang et al. [92] exposed male Guinea pigs (n=10 per group) to 3500 MHz EMF at an average SAR of 2, 4 or 10 W/kg for 72 h. Hearing thresholds and behaviour did not change after exposure. Malondialdehyde levels in the auditory cortex were increased and catalase, superoxide dismutase and glutathione peroxidase activities were decreased in the exposed groups compared to the sham-exposed group. They also observed ultrastructural changes in the auditory cortex, including swollen mitochondria and layered myelin sheaths, as well as cytochrome-c re-localization and activation of caspase-9 and cleaved caspase-3. They suggest that exposure resulted in increased oxidative stress, but this did not result in effects on hearing and behaviour.

Qin et al. [93] exposed C57Bl/6 mice (n=12 per group) to 4.9 GHz EMF for 1 h per day during 21 days. The power density was 50 W/m^2. After exposure, anxiety-like behaviour and spatial memory ability of the exposed mice was not different from that in sham-exposed animals, but depression-like behaviour was induced in the exposed group. Also, the number of neurons in the amygdala was significantly reduced and the level of pyroptosis (a form of cell death that is triggered by proinflammatory signals and associated with inflammation)was increased. No such changes were observed in the hippocampus.

4.3.3 Cancer

Cappucci et al. [42] exposed the fruit fly *Drosophila melanogaster* to 2.4 GHz EMF continuously during development at a power density of 0.0048 W/m^2.They observed extensive heterochromatin decondensation and thus a general loss of transposable elements epigenetic silencing in both germinal and neural tissues. No effect was found on Hsp70 in various larval tissues, but genomic instability

occurred in larval brains, as well as increased oxidative stress. Locomotor behaviour in both larvae and adults was impaired. Finally, they investigated tumour promotion in animals that were genetically modified to overexpress a tumour-associated gene, RasV12, in the developing eye. In sham-exposed larvae, clones of cells expressing this gene showed benign overgrowth and rarely invaded other tissues, while in real exposed animals, tumour overgrowth and metastases occurred.
A SAR of 0.0608 W/kg was mentioned, but no information was given how this was assessed.

4.3.4 Development

Sarapultseva et al. [94] exposed the water flea *Daphnia magna* (n=48-56 per group) to 900 MHz EMF at a power density of 1 mW/cm^2 (10 W/m^2). Exposure was continuously during the juvenile (120 h from day 1-5 after birth) and pubertal periods (120 h from day 5-10 after birth). They observed no effect of exposure on survival. A decrease in fertility was observed, which was strongest in the animals exposed as juveniles. Also, the size of the exposed animals was reduced, with no difference between the two age groups. These effects were not found in subsequent generations. Cytotoxicity was increased in the animals treated as juveniles, but decreased compared to controls in the animals exposed at a later age.

De Paepe et al. [95] exposed pupae (n=200 per group) of the Blue Bottle Fly (*Calliphora vomitoria*) to 5.4 GHz EMF for 48 h at a SAR level of 0.028 or 0.23 W/kg. No effect on pupal mass, length and diameter was observed. In the highest exposure group, adult flies hatched earlier relative to the sham and lower exposed groups.

Yildirim et al. [96] exposed pregnant Balb/c mice to 10.715 GHz for 12 hours per day until birth at a whole-body SAR of 0.725 W/kg. Total RNA and cDNA were obtained from brain tissue of newborn mice (n=12 control, n=29 exposed). Alterations in the expression of 7 genes known to have a role in neuronal migration were investigated. The expression of 6 of these genes, ARX, FLNA, DCX, LARGE, RELN and YWHAE,) was found to be significantly increased. What the effect of these changes is in terms of neuronal migration and development is not clear.

Yan et al. [97] exposed male C57BL/6J mice to 2.0 GHz EMF continuously for 10 weeks at a whole-body SAR of 0.125-0.5 W/kg. An elevated apoptosis rate in testis germ cells was observed, but no effects on testis organization, sperm quality and pregnancy rate. Also, reduced weight and altered glucose metabolism were observed in male, but not in female offspring. This was associated with a decrease in the expression in the liver of a glycolytic gene (Gck), while the expression of another glycolytic gene (Pklr), two gluconeogenic genes (G6pc and Pck1) and a glycogenolytic gene (Pygl) were not changed.

Sun et al. [98] exposed the flatworm *Caenorhabditis elegans* to 9.4 GHz high-power microwaves with repetition frequencies of 10, 20, and 50 Hz for 30 min at SAR levels of 4.33, 8.66 or 21.65 W/kg. They investigated development, movement, egg production, oxidative stress parameters and lifespan at different times after exposure. They did not observe any differences with sham-exposed groups.

In a second paper of this group, Sun et al. [99] exposed the flatworm *Caenorhabditis elegans* to a continuous 9.4 GHz EMF at a SAR level of 4 W/kg. The first generation was exposed for 10 h per day, but the subsequent 20 generations were continuously exposed. The 10th, 15th and 20th generations were investigated. From the 10th generation on, the growth of the animals decreased, their motility decreased and oxidative stress was measured. From the 15th generation, fecundity also decreased.

4.3.5 Fertility

Pardhiya et al. [100] exposed male Wistar rats (n=6 per group) to 2002 MHz EMF for 2 h per day, 6 days per week during 8 weeks, at a whole-body SAR of 1.2 W/kg. Compared to sham-exposed controls, real exposure resulted in a decrease in weight of the testis, seminal vesicles and prostate, but no effect on epididymis weight. Exposure resulted also in a decrease in sperm count and in increase in damaged sperm. Testosterone was decreased, but no effect was found on luteinizing and follicle stimulating hormones. Histological examination showed a decrease in germinal epithelium height and the diameter of seminiferous tubules, but not on the thickness of the tunica albuginea. Oxidative stress in the testis was increased.

Er et al. [101] exposed male Wistar rats to 900 MHz EMF (n=6 per group) for 2 h per day, 5 days per week during 1 or 10 weeks. The testis SAR was 0.107 W/kg. No histological abnormalities were observed. The expression of cleaved caspase-3, indicative of apoptosis, was not different between 1-week-exposed and sham-exposed groups, but a reduction was observed in the 10-week-exposed group. On the other hand, in the short-exposed group a reduction was observed in the level of Bcl-xL, an anti-apoptotic protein, but not in the longer-exposed group. In addition, both p-p38 and p-JNK protein expressions, indicative of oxidative stress, increased significantly in both exposed groups.

Soleimani et al. [102] exposed male NMRI mice (n=7-8 per group) to 950 MHz EMF from a mobile phone in standby mode kept at a distance of 5 or 20 cm. The measured power density averaged over 6 min was 3.11 and 1.49 $\mu W/m^2$, respectively, with the level at 20 cm being similar to the background level without a phone present. The animals were exposed while loosely restrained for 6 h per day, 5 days per week during 6 or 10 weeks. Testis diameter was slightly reduced in both groups kept at 20 cm. The external diameter of seminiferous tubules was increased in the 5 cm group at 6 weeks and decreased in the 5 and 20 cm group at 10 weeks. The internal diameter of the seminiferous tubules was increased in the 5 cm group at 6 weeks and decreased in the 20 cm group at 6 and 10 weeks. The height of the germinal epithelium was increased in the 5 cm group at 6 weeks and decreased at 10 weeks. Sperm counts were increased in the 20 cm group at 6 weeks. Sperm motility was decreased in the 5 cm group at 10 weeks and increased in the 20 cm group at 6 and 10 weeks. The percentage of non-motile sperm was increased in the 5 cm group at 10 weeks.

Xue et al. [103] exposed male Sprague Dawley rats (n=18 per group) to 5.8 GHz EMF for 1 h per day during 30 days. The testis SAR was 3.36 W/kg. No significant differences were observed in sperm count and sperm abnormalities, and in the levels of testosterone, follicle-stimulating hormone, luteinizing hormone, glial cell line-derived neurotrophic factor, stem cell factor and the apoptosis-related protein caspase-3.

Gupta et al. [104] exposed two-week-old male chickens (n=7 per group) to 2.45 GHz EMF for 2 h per day during 30 days at a whole-body SAR of 0.9978 W/kg. They found a decreased testicular weight, volume and gonado-somatic index, as well as a reduction in the diameter of seminiferous tubules. Also, an increase in oxidative stress parameters in the testes was observed and an increase in IL-1β immunoreactivity, a decline in IL-10 immunoreactivity and a decrease in oestrogen receptor alpha expression, indicating inflammatory stress.

4.3.6 Oxidative stress

Salameh et al. [105] exposed female Sprague-Dawley rats (n=16 per group) pre- and postnatal to 900 MHz EMF, 24 h per day, from gestational day 1 to day 21 after birth. The exposure at an E-field level of 25 V/m [they calculated a SAR, but using the external instead of internal E-field]. They assessed oxidative stress and other parameters in the liver at postnatal days 1, 9 and 21. They found changes in all parameters at all ages, indicating increased oxidative stress.

Sharma et al. [106] exposed male Wistar rats (n= 10 per group) to 2100 MHz EMF for 4 h per day, 5 days per week during 3 months. The whole-body SAR was 0.453 W/kg. Increased levels of triglycerides and cholesterol in serum were observed, as well as changes in glutathione homeostasis followed by an activated inflammatory response. Histopathology showed a change in the myelination pattern and cellular organelles in the brain.

Kucukbagriacik et al. [107] exposed male Swiss-Albino mice (n=6 per group) to 900 MHz EMF for 4 h per day during 7 days, at a whole-body SAR of 0.269 W/kg. No effect was observed on reactive oxygen species and malondialdehyde in plasma, but catalase, superoxide dismutase and total antioxidant capacity in plasma were decreased. This was also the case for the expression of several genes involved in DNA repair in the liver, but apoptosis in the liver was not changed.

Tomruk et al. [108] exposed pregnant and non-pregnant New Zealand White rabbits to 1800 MHz EMF, 15 min per day for a week from day 15 to 22 of gestation. The whole-body SAR was 2 mW/kg. After birth, both adults (pregnant and non-pregnant) and newborns were sacrificed, and their livers removed for analysis (n=9 per group). Two regulatory enzymes in the oxidative phase of phosphogluconate pathways (glucose-6-phosphate dehydrogenase and 6-phosphoglucose dehydrogenase) were analysed to interpret the biosynthesis of cytosolic NADPH for maintaining mitochondrial energy metabolism. Moreover, the efficiencies of maternal glutathione-dependent enzymes (glutathione S-transferase, thioredoxin, glutathione peroxidase and glutathione reductase) on both the removal of metabolic disturbances during pregnancy and foetus development were examined. Glucose-6-phosphate dehydrogenase, 6-phosphoglucose dehydrogenase, glutathione S-transferase and thioredoxin were decreased in the exposed non-pregnant adults, but not in the exposed pregnant adults compared to the sham-exposed groups. Glutathione peroxidase and glutathione reductase were also decreased in the non-pregnant exposed animals, but increased in the pregnant ones. In the newborns, no significant differences in these enzymes were observed between real and sham-exposed groups.

4.3.7 Heart

Yavaş et al. [109] exposed male Sprague Dawley rats (n=7 per group) to 2100 MHz EMF for 5 h per day during 14 days. The SAR in the heart was 0.234 W/kg and in the spleen 0.209 W/kg. Histopathological and immunohistochemical evaluation revealed no significant changes in heart tissue, but trabecular irregularity and enlargement of sinusoids were observed in the spleen.

Bozok et al. [110] exposed pregnant Sprague Dawley rats (n=3 per group) to 900, 1800 and 2100 MHz for 6, 12 or 24 h over 20 days, at a whole-body SAR of 0.17 W/kg. Histopathological and biochemical analysis of myocardial tissue of newborn pups (n=6 per group) was performed. In the animals exposed to 1800 MHz or 2100 MHz, more myocardial damage, an increased malondialdehyde level and a decreased glutathione level were found in the 24-h exposed groups compared to the groups exposed for shorter times. No changes were observed in the animals exposed to 900 MHz, but reporting of this group is incomplete.
The main data analysis was done as a function of frequency, which is not correct.

Zhang et al. [111] exposed male Wistar rats (n=5 per group per assay) to 2.856 GHz at a whole-body SAR of 1.7 or 3.3 W/kg, to 1.5 GHz at a SAR of 1.8 or 3.7 W/kg, all for 6 min, or to 2.856 GHz for 6 min followed by 1.5 GHz for another 6 min, at the lower or higher SAR levels. They observed changes in myocardial functional parameters and ECG, that were more severe at 6 h and at 7 days following exposure, but that subsided at later assay points, 14 and 28 days after exposure. In general, the changes were stronger in the groups exposed to the highest SAR levels, but there were no differences between the frequencies. The groups treated with both frequencies in general showed stronger responses than the ones exposed to a single frequency. They also assayed in cardiomyocytes the proteins Beclin-1 and LC3, that are indicative of cardiomyocyte injury. Both proteins were significantly increased 7 days after exposure in the groups exposed to the highest SAR Levels, especially in the group treated with both frequencies.

4.3.8 Other endpoints

Yao et al. [112] exposed male Wistar rats (n=20 per group) to 2.8 or 9.3 GHz, or both frequencies sequentially, for 6 min per frequency at a power density of 10 mW/cm^2 (100 W/m^2). Each treatment resulted in alterations in the thymus and spleen at 7 days after exposure, which were more pronounced when both frequencies were applied. The latter treatment led to an increase in the number of leukocytes and lymphocytes in peripheral blood and an increase in the proportion of B lymphocytes. Cytokines associated with the proliferation and activation of B lymphocytes, including interleukin (IL)-1α, IL-1β and IL-4, were elevated at 6 hours after both single and combined frequency exposure. Combined, but not single frequency exposure upregulated the mRNA and protein expression of B cell activation-associated genes in peripheral blood. expression of genes related to DNA duplication, cellular metabolism and signal transduction in the peripheral blood and spleen.

In a study described earlier under effects on the brain, Tan et al. [113] exposed Wistar rats to 2450 MHz EMF for 12 h per day at a power density of 8.5 ± 0.65 W/m^2, starting at 1 month before fertilization and continuing into 4 generations. They exposed either males, females or both. When animals of the 2nd, 3rd and 4th generation were 2 months old, they were sacrificed and their thymuses investigated. No changes could be distinguished in both males and females of the 2nd and 3rd generation, but vascularization was observed in the thymus of the 4th-generation offspring of the group where both males and females were irradiated. They also observed that the number of offspring and mass of all rats decreased in the 3rd-generation group.

Kim et al. [114] exposed male Sprague Dawley rats (n=10 per group) to 1760 MHz EMF for 6 h, at an whole-body SAR of 4 W/kg. They measured body temperature by either rectal thermometry or implanted iButtons. There was a good correlation between the two types of measurement and the changes in body temperature were less than 1°C.

Leberecht et al. [115] exposed the night-migratory songbird Eurasian blackcap to broadband 75-85 MHz EMF at a spectral density of 2.53 pT/√Hz in an otherwise shielded environment. In behavioural experiments, the exposure prevented the birds from using their magnetic compass. They speculate on the mechanism, that at least one of the components of the radical pair involved in the sensory process of avian magnetoreception must contain a substantial number of strong hyperfine interactions, as would be the case if a flavin-tryptophan radical pair were the magnetic sensor.

In an observational study, Nath et al. [116] counted House Sparrows and Tree Sparrows in Guwahati City, India, during 2 years and correlated their numbers with several environment factors, including EMF (broadband measurements in the range 50 MHz – 3.5 GHz were made). No correlation with EMF-RF was found.

4.3.9 Conclusions

As in previous years, there is again a variety of endpoints and exposure parameters, such as frequency, duration and exposure level. Also as in previous years, most included studies show some effects of exposure, and a few do not. Effects are observed in all endpoints considered: effects on the brain, cognition and behaviour, cancer, development, fertility, oxidative stress, effects on the heart and several other endpoints. Effects are more often observed at relative high, but also at extremely low exposure levels. Therefore, general conclusions on effects of RF EMF exposure in experimental animals cannot be drawn based on the studies of 2022, but it is possible that under certain circumstances effects are induced on different endpoints. It would be better to analyze the data in a different way, per endpoint and including all available data over time. This is currently being done by WHO, and that analysis includes several systematic reviews of major endpoints, such as carcinogenicity and adverse reproductive outcomes. There is currently disagreement among scientists on whether RF EMF exposure can induce carcinogenic or other effects in animals. The outcome of an ongoing, systematic analysis by WHO, which is expected in 2024, might shed a clearer light on this.

Out of the 68 retrieved studies, 26 had to be excluded from analysis because of various reasons. It is still of concern that many of these studies had to be excluded because of a flawed study design (mainly no sham-exposed group, or only very small groups of animals) or missing crucial information on dosimetry. Analyses that include all studies regardless of their quality will provide a biased picture.

Table 4.3: Animal studies RF-EMF

Endpoint	Reference	Species	Exposure and duration	Results
Effects on brain	Souffi et al. [77]	Wistar rats, male	1800 MHz, 2 h; brain SAR 0.5 W/kg	Animals with neuroinflammation: effects on auditory system; healthy animals: no effects.
	Orlacchio et al. [78]	C57Bl6 mice, female	1.8 GHz, 210 sec; brain SAR 2 or 6 W/kg	No changes in time course of evoked functional ultrasound response in cortex.
	Liu et al. [79]	Sprague Dawley rats, male	9.37 MHz, pulsed @ 1.875 Hz, 2 min; WBA SAR 6.08 W/kg	Impaired spatial learning and memory; attenuated by 20-hydroxyecdysone, which facilitated neurogenesis.
	Dasdag et al. [80]	Wistar rats, male	2.4 GHz WiFi, 24 h/d, 1 year; WBA SAR 0.000328 W/kg, brain SAR 0.000655 W/kg	Increased expression of microRNA rno-miR-181a-5p and of phosphatidylserine and triacylglycerol.
	Tümkaya et al. [81]	Sprague Dawley rats, female	900 MHz, during pregnancy; WBA SAR 0.087 W/kg	In offspring, no effect auditory brainstem responses; damage in cochlear nuclear neurons and oligodendrocyte cell structures, increased caspase-3 activity.
	Tan et al. [82]	Wistar rats, male and female	2450 MHz, 12 h/day, continuous during 4 generations; brain SAR 0.071 W/kg	In all generations, haemorrhagic areas, irregular cellular localization and vascular structures in brain of foetal and adult female rats, increased levels of pERK, ptau, pJNK and pP38 in brain of adult male rats.

	Bektas et al. [83]	Wistar rats, male	3.5 GHz, 2 h/d, 30 d; brain SAR 0.00195 W/kg	Increased oxidative stress in brain. In brain increased ghrelin, decreased nesfatin-1 (hormones, influence food intake), increased irisin (hormone, potential in treatment obesity, diabetes).
	Kopani et al. [84]	New Zealand White rabbits, sex not given	1805-1870 MHz, 150 min, 300 V/m and/or 1788 MHz, 50% pulse modulated, 150 min, 160 V/m	No effect iron concentration in cerebellum (indicator of disruption of blood-brain barrier).
Cognition and behaviour	Perov et al. [18]	Wistar rats, male	3.6, 28 and 37 GHz, continuously 4 mo; 250 µW/cm^2	Assays at the end of each month of exposure and 1 month after exposure. After 2 months exposure decrease in exploratory activity, at 1 month after exposure back to normal. No effect on ACTH, corticosterone increased at 1 and 2 months during exposure and at 1 month after, but not in between these assay times.
	Li et al. [85]	Wistar rats, male	2.856 GHz, 6 min, 3x/wk, 6 wk; power density 30 mW/cm^2	Rats carrying rs198585630 C allele: cognitive deficits and inhibition of brain electrical activity. Possibly thermal effect.
	Dasgupta et al. [86]	Zebrafish embryos	3.5 GHz, 6-48 h post-fertilization; WBA SAR 8.27 W/kg	Increased avoidance response to predators decreased schooling response to other zebrafish; transcriptomic disruption directly after exposure (28 differentially expressed genes); biochemical pathways related to metabolism perturbed.
	Vargová et al. [87]	*Ixodes ricinus* ticks, female	900 MHz, 25 h, 1 mW/m^2	No differences in preference for irradiated and shielded parts of tube, time spent and trajectory passed significantly longer in exposed part of open field arena.
	Hao et al. [88]	C57BL/6N mice, male	300 kV/m electromagnetic pulses with center frequency 100 MHz, 400 pulses at 1 Hz	Neuronal activity in hippocampus inhibited during exposure, recovered immediately after. At 1 day after exposure decreased neuronal activity, 7 days after exposure some recovery. Damaged structures in hippocampal neurons.
	Aeen et al. [89]	Sprague Dawley rats, male	2.45 GHz, pulse modulated 500 Hz, 45 min/d, 4 or 8 d; power density 4 mW/cm^2	Decreased spatial memory, increased anxiety. 8 d-exposed: decreased serum globulin, no effect albumin and total serum protein, increased reaction time in hot plate test.

	Wang et al. [90]	Wistar rats, male	1.5 GHz, WBA SAR 3.3 W/kg and/or 4.3 GHz, WBA SAR 2.5 W/kg, 6 min	Impaired spatial learning and memory, decreased cortical excitability, damage to hippocampal ultrastructure in all groups. Differentially expressed proteins involved in synaptic vesicle cycle and vesicular transport.
	Wang et al. [91]	Wistar rats, male	1.5 GHz, 6 min; brain SAR 1.85, 11.1, 18.5 W/kg	Maximum core temperature increase ~1 °C. Two highest SAR levels: exposure level-dependent impaired spatial memory, inhibited EEG activity, pyknosis and hyperchromatism of neuron nucleus, changes in NMDAR subunits and downstream signalling molecules. Local thermal effect possible.
	Yang et al. [92]	Guinea pigs, male	3500 MHz, 72 h; WBA SAR 2, 4, 10 W/kg	No effect hearing thresholds and behaviour. Increased oxidative stress, ultrastructural changes in auditory cortex.
	Qin et al. [93]	C57Bl/6 mice, male	4.9 GHz, 12 h/d, 21 d; power density 50 W/m^2	No effect anxiety, spatial memory. Induced depression-like behaviour. Number of neurons in amygdala reduced, level of pyroptosis increased. No changes in hippocampus.
Cancer	Cappucci et al. [42]	Fruit fly *Drosophila melanogaster*	2.4 GHz, continuously during development; power density 0.0048 W/m^2	Heterochromatin decondensation in germinal and neural tissues. No effect Hsp70 in various larval tissues. Genomic instability, increased oxidative stress in larval brains. Tumour promotion in genetically modified animals.
Development	Sarapultseva et al. [94]	Water flea *Daphnia magna*	900 MHz, continuously during the juvenile (120 h from day 1-5 after birth) and pubertal periods (120 h from day 5-10 after birth); power density 10 W/m^2	No effect on survival. Decrease in fertility, strongest in animals exposed as juveniles. Reduced size, no difference between age groups. No such effects in subsequent generations. Cytotoxicity increased in animals treated as juveniles, decreased in animals prepubertal exposed.
	De Paepe et al. [95]	Blue Bottle Fly (*Calliphora vomitoria*) pupae	5.4 GHz, 48 h; SAR 0.028, 0.23 W/kg	No effect on pupal mass, length and diameter. Adult flies in highest SAR group hatched earlier than sham and lower exposed groups.
	Yildirim et al. [96]	Balb/c mice, female	10.715 GHz, 12 h/d during pregnancy; WBA SAR 0.725 W/kg	Increased expression of 6/7 genes involved in neuronal migration.

	Yan et al. [97]	C57BL/6J mice, male	2.0 GHz, continuous, 10 wk; WBA SAR 0.125-0.5 W/kg	Elevated apoptosis rate in testis germ cells, no effects on testis organization, sperm quality, pregnancy rate. Reduced weight, altered glucose metabolism in male, not in female offspring. Associated with decrease in expression in liver of glycolytic gene Gck, expression of glycolytic gene Pklr, two gluconeogenic genes (G6pc and Pck1) and glycogenolytic gene (Pygl) not changed.
	Tomruk et al. [108]	New Zealand White rabbits, female (pregnant / non-pregnant)	1800 MHz, 15 min/d, 1 wk, d 15-22 of gestation; WBA SAR 2 mW/kg	Liver enzymes glucose-6-phosphate dehydrogenase, 6-phosphoglucose dehydrogenase, glutathione S-transferase and thioredoxin decreased in non-pregnants, not in pregnants. Glutathione peroxidase, glutathione reductase decreased in non-pregnants, increased in pregnants. No such effects in newborns.
	Sun et al. [98]	Flatworm *Caenorhabditis elegans*	9.4 GHz high-power, repetition frequencies 10, 20, 50 Hz,30 min; SAR 4.33, 8.66 or 21.65 W/kg.	No effect on development, movement, egg production, oxidative stress parameters and lifespan.
	Sun et al. [99]	Flatworm *Caenorhabditis elegans*	9.4 GHz, 1st generation 10 h/d, next generations continuous; SAR 4 W/kg	From 10th generation on, growth, motility decreased, oxidative stress measured. From 15th generation, fecundity decreased.
Fertility	Pardhiya et al. [100]	Wistar rats, male	2002 MHz, 2 h/d, 6 d/wk, 8 wk; WBA SAR 1.2 W/kg	Testis, seminal vesicles, prostate weight decreased, no effect epididymis weight. Sperm count decreased, damaged sperm increased. Decreased testosterone, no effect luteinizing and follicle stimulating hormones. Germinal epithelium height tubule diameter decreased, no effect tunica albuginea. Increased oxidative stress in testis.
	Soleimani et al. [102]	NMRI mice, male	950 MHz, 6 h/d, 5 d/wk, 6 or 10 wk; power density 3.11 or 1.49 µW/m2	Various effect on testis morphology and sperm observed, but no consistent relation with exposure level and time.
	Er at al. [101]	Wistar rats, male	900 MHz, 2 h/d, 5 d/wk, 1. 10 wk; testis SAR 0.107 W/kg	No histological abnormalities. Decreased cleaved caspase-3 (indicative of apoptosis) in 10-wk-exposed. Decreased Bcl-xL (anti-apoptotic protein) in 1-wk-exposed. Increased oxidative stress in both groups.
	Xue et al. [103]	Sprague Dawley rats, male	5.8 GHz, 1 h/d, 30 d; testis SAR 3.36 W/kg	No effects on testis.

	Gupta et al. [104]	Chickens, male	2.45 GHz, 2 h/d, 30 d; WBAS SAR 0.9978 W/kg	Decreased testis weight, volume, gonado-somatic index, tubule diameter. Increased oxidative stress, inflammatory stress.
Oxidative stress	Salameh et al. [105]	Sprague-Dawley rats, female	900 MHz, 24 h/d, gestational d1 to d21 after birth; E field 25 V/m	Increased oxidative stress in liver at 1, 9 and 21 d after birth.
	Sharma et al. [106]	Wistar rats, male	2100 MHz, 4 h/d, 5 d/wk, 3 mo; WBA SAR 0.4523 W/kg	Increased triglycerides, cholesterol levels in serum, changes in glutathione homeostasis, inflammatory response. Change in myelination pattern and cellular organelles in brain.
	Kucukbagriacik et al. [107]	Swiss-Albino mice, male	900 MHz, 4 h/d, 7 d; WBA SAR 0.269 W/kg	No effect reactive oxygen species, malondialdehyde in plasma, catalase, superoxide dismutase, total antioxidant capacity in plasma decreased. Decreased expression of several genes involved in DNA repair in liver, apoptosis in liver not changed.
Heart	Yavaş et al. [109]	Sprague Dawley rats, male	2100 MHz, 5 h/d, 14 d; heart SAR 0.234 W/kg, spleen SAR 0.209 W/kg	No effect in heart. Trabecular irregularity, sinusoid enlargement in spleen.
	Bozok et al. [110]	Sprague Dawley rats, female, pregnant	900, 1800, 2100 MHz, 6, 12, 24 h over 20 d; WBA SAR 0.17 W/kg	Newborns, 1800, 2100 MHz, 24 h exposed: more myocardial damage, increased oxidative stress than other groups.
	Zhang et al. [111]	Wistar rats, male	2.856 GHz, WBA SAR 1.7, 3.3 W/kg; 1.5 GHz, WBA SAR 1.8, 3.7 W/kg; 6 min each, or sequentially	Changes in myocardial functional parameters and ECG, stronger in highest exposed groups, no differences between frequencies. Increased cardiomyocyte injury proteins in highest exposure groups.
Other endpoints	Yao et al. [112]	Wistar rats, male	2.8, 9.3 GHz, 6 min; power density 100 W/m^2	Alterations in thymus and spleen 7 d after exposure, increased stimulation immune parameters.
	Tan et al. [113]	Wistar rats, male and female	2450 MHz, 12 h/day, continuous during 4 generations; brain SAR 0.071 W/kg	No effect on thymus in 2nd, 3rd generation, vascularization in 4th generation. Number of offspring decreased in 3rd generation.
	Kim et al. [114]	Sprague Dawley rats, male	1760 MHz, 6 h; WBA SAR 4 W/kg	Core body temperature changed less than 1°C.
	Leberecht et al. [115]	Night-migratory songbird Eurasian blackcap	75-85 MHz; spectral density 2.53 pT/√Hz	Prevention from using magnetic compass.
	Nath et al. [116]	House Sparrows and Tree Sparrows	Environmental EMF	No correlation population numbers with EMF levels.

4.4 Cell studies

4.4.1 Cell transformation

Ding et al. (2022) [117] exposed Balb/c-3T3 mouse fibroblasts to 1800 MHz CW, 8 W/kg SAR, 4 h per day for 40 days and 60 days to evaluate malignant transformation. Cells were harvested for a cell transformation assay, transplantation in SCID mice, and a trans-well assay.
The results of three independent experiments, carried out double blind and with triplicate samples, indicated malignant transformation, evaluated as transformed foci, in the 40-day ($p<0.05$) and 60-day ($p<0.001$) exposed groups compared to sham controls. The authors also reported an increased cell proliferation in exposed samples, evaluated as migration, although no p-value was reported.

To evaluate the capability to induce tumours, SCID mice were transplanted with exposed Balb/c-3T3 cells. No tumours were seen in mice transplanted with sham cells, while animals transplanted with cells exposed for both 40 and 60 days showed visible tumours, evaluated according to their size and weight. Transplantation with HeLa cells was used as positive control and to compare the induced carcinogenicity.
Clone formation also increased, indicating a RF-induced increase in cell growth, but also in this case no p-value was reported.
To identify the key genes and signalling pathways involved in the detected effects, the mRNA microarray was used and, based on the significantly expressed genes, the authors concluded that lipid metabolism was the main involved biological process.

4.4.2 DNA methylation

Ergun et al. [118] evaluated the effect of 2 h exposure to 2100 MHz, 2 W/kg SAR, on C3H cancer mouse fibroblasts, in terms of viability and activation of Nuclear factor kappa (NFKB) and DNA methyltransferase (DNMT), two enzymes involved in the regulation of DNA methylation. The possible protective effect of Zinc (Zn), a metal involved in the modulation of epigenetic mechanisms, was also evaluated by exposing cell cultures in absence and in presence of different concentrations of Zn.
In six independent experiments, a slight but statistically significant increase in cell viability was detected in exposed cells, compared to sham-exposed ones ($p <0.05$). Such an increase was not induced in samples exposed in presence of Zn. NFKB and DNMT activity also increased with RF exposure ($p<0.001$) and was reduced with the Zn treatments. The experiments were not performed blinded. (Ergun et al., 2022). As a whole, the results suggest that the effect of RF exposure could be reduced by Zn treatments.

4.4.3 Neuronal functions

Changes in neuronal excitability, intracellular calcium levels and synaptic transmission were investigated by Echchgadda et al. [119] in primary hippocampal embryonic rat neurons (PHNs) following 1 h exposure to 3 GHz RF-EMF at an average SAR of ~0.3 W/kg (maximum SAR of ~0.7 W/kg). During the exposure, temperature was monitored and the difference never exceeded 0.2°C by comparing exposed and sham exposed samples.
Neuronal excitability, measured as evoked action potential (eAP) by whole cell patch-clamp recordings, resulted in a statistically significant decrease in the amplitude of eAPs in exposed versus unexposed cells ($p = 0.03$) in 20 measurements. A significant ($p = 0.03$) depolarization of the resting membrane potential (MP) after exposure was also detected in 16 measurements, suggesting that RF exposure could trigger PHNs to be more excitable. The analysis also revealed a slight increase of AP

duration and a more positive AP firing threshold in the exposed neurons compared to sham controls. Regulation of calcium is a major mechanism by which changes in MP can control neurophysiological processes such as cell excitability, neurotransmitter release, and synaptic plasticity and is AP dependent. A significant increase in basal $Ca2+$ level was observed at 15 min post ($p < 0.001$) in 48 measurements, suggesting a prolonged increase of neuronal excitability.
To assess the effect of 3.0 GHz RF-EMFs on synaptic transmission, postsynaptic excitatory and inhibitory currents were recorded following sham and RF exposures and the results indicated an increase in synaptic activity.
No significant changes in plasma membrane (PM) resistance and in cell proliferation were detected. The authors concluded that their results do not provide sufficient evidence to support nor to refute a RF-EMF non-thermal mechanism of interaction.

4.4.4 Autophagy

Autophagy is a cytoprotective program whose purpose is to help eucaryotic cells to adapt to any challenging situation. It is the first line of defence against physical, nutritive, or chemical stress, exposure to infections or by intracellular alterations. Therefore, in response to a wide array of stimuli, the autophagic program, which normally runs at a basal level to warrant intra-cellular quality control, will be up-regulated. Autophagy-related genes (ATG) will orchestrate the increased turnover of dispensable cellular components to synthesize those essential for cell survival. Vesicular trafficking will thus be intensified, where portions of cytoplasmic constituents and organelles are enclosed in autophagosomes and routed to release their cargo into lysosomes. The resulting degradation products are further made available to be reused for the synthesis of essential components.

In the period of interest, two papers have been published to evaluate the involvement of autophagy in the cellular response to RF exposure.

Hao and co-workers [120] evaluated the induction of autophagy in rat adrenal pheochromocytoma (PC12) cells exposed for 15 min to 2856 MHz at an average power density of 30 mW/cm2, corresponding to a calculated SAR of approximately 19.0 W/kg (Hao et al., 2022). To this purpose, the level of the autophagic marker LC3-II was measured in cultures pre-treated with or without chloroquine (CQ), a lysosomal inhibitor. In the CQ-untreated cells, the LC3-II content showed no difference between the sham and exposed samples. In samples treated with CQ and exposed to RF, the expression of LC3-II was increased at 6 h ($p<0.05$) but restored to basal levels at 12 and 24 h after exposure, suggesting an enhanced ‘autophagic flux’ induced by RF exposure. In addition, the expression of several autophagic markers was also measured: Beclin1 was up-regulated at 6 h and restored at 12 h post-exposure, and autophagic genes (Atg) Atg5, Atg7 and Atg9 were up-regulated at 6 and 12 h after exposure ($p<0.05$), suggesting the enhanced expression of autophagy-related genes induced by RF exposure.
Autophagosomes are double-membrane vesicles newly formed during autophagy to engulf a wide range of intracellular material to be transported to lysosomes (autolysosomes). The authors observed an increased number of autophagosomes and autolysosomes at 6 h after exposure in cultures pretreated with CQ at 6 h after exposure.
It is known that microRNAs (miRNAs) play important roles in physiological and pathological events in the central nervous system. In this study, the authors also detected the down-regulation of miR-30a (an RNA gene participating in many cellular processes) induced by RF exposure and an increased expression of Adenosine monophosphate (AMP)-activated protein kinase-α2 (AMPKα2) and the activation of AMPK signalling, which promoted the occurrence of autophagy and the biogenesis of autophagosomes.
They concluded that, taken together, the results suggest that under the adopted experimental

conditions, functional autophagy was activated by RF exposure. However, it must be underlined that in this study a) the SAR value employed is much higher than the one set by the regulatory limits and b) the experiments have been carried out at room temperature, without a temperature control.

In the last decade, several papers have been published by Sannino and co-workers, where it was evidenced that mammalian cells pre-exposed to RF were protected from the damage induced by a subsequent treatment with chemical or physical genotoxic agents. In a paper published in 2022 the research group evaluated whether autophagy was involved in such a phenomenon (Sannino et al. [121]).

To this purpose, SH-SY5Y human neuroblastoma cells were exposed for 20 h to 1950 MHz, UMTS signal, 0.3 W/kg SAR, and then treated with menadione (MD), a chemical agent inducing oxidative stress and oxidative DNA damage.

In three to four (depending on cell type) independent experiments carried out by applying the comet assay, they confirmed the absence of effects after exposure to RF alone and the reduction of MD-induced DNA damage when RF exposure was given before the chemical treatment. In addition, the protective effect of RF-EMF pre-exposure was negated when autophagy was blunted by chemical inhibitors (treatments with bafilomycin A1 and E64d) and as a result of the CRISPR-driven genetic edition of ATG genes. The exposure was carried out blinded and the temperature never exceeded the instrument sensitivity (±0.3 °C). The negation of protection induced by RF-EMF following both chemical and genetic inhibition of autophagy, which strengthen each other, suggest a possible role of this catabolic cellular pathway in the observed phenomenon.

4.4.5 Other cellular endpoints

In a paper by Joushomme et al. [122], the global cellular stress response to RF-EMF exposure was evaluated with label-free techniques, in real time, using impedancemetry (xCELLigence set-up) and Digital Holographic Microscopy (DHM). Impedance changes reflected variations in cell morphology (size, volume, shape, or spreading), cell number (proliferation or death), or movement (migration or extravasation) and were reported in a dimensionless parameter called Cell Index (CI). Exposures of attached cells to RF-EMF signals and impedance CI measurements were done simultaneously through the gold electrodes of the xCELLigence plates. DHM images were obtained using a HoloMonitor M4. The RF-EMF exposure system was a tri-plate open transverse electromagnetic (TEM) cell allowing RF-EMF signals propagation. Both TEM cell ground plates had apertures to allow for light propagation through the cell culture. Average cell area, cell volume, and cell thickness were assessed. Exposures to RF-EMF at 1800 MHz alone or during co-exposure with chemical treatments known to induce either apoptosis (Arsenic trioxide As2O3) or autophagy (serum-deprivation using serum-free Hanks'Balanced Salt Solution HBSS) were tested. Two human cell lines, neuroblastoma (SH-SY5Y) and colorectal carcinoma (HCT116) cells and two cultures of primary cells from rat cortex (astrocytes and co-cultures of neurons and glial cells) were exposed to RF using a carrier wave at 1800 MHz modulated with various environmental signals: GSM (Global System for Mobile Communications, 2G signal), UMTS (Universal Mobile Telecommunications System, 3G signal), LTE (Long-Term Evolution, 4G signal) and Wi-Fi or unmodulated RF-EMF (continuous wave, CW). The SARs used were 1.5 and 6 W/kg for the DHM experiments and varied from 5 to 24 W/kg for the impedancemetry. Cell cultures were exposed continuously for three to five consecutive days, maintaining the temperature at 37°C. The results indicate that exposure to RF-EMF, if compared to sham-exposed conditions, would have no impact on the overall behaviour of healthy cells or cells engaged in an apoptotic or autophagic process, for all the experimental conditions tested.

Table 4.5: Cell studies on exposure to radiofrequency fields

Cell type	Endpoint	Exposure conditions	Effect	References
Mouse fibroblasts (Balb/c-3T3) n = 3	Transformation (foci, migration, mRNA microarray)	1800 MHz, CW 8 W/kg 40 and 60 days (4h/day)	Transformed foci formation, Increased cell proliferation, induction of tumour formation in transplanted mice, increased expression of genes involved in lipid metabolism.	Ding et al. (2022)
Cancer mouse fibroblasts (C3H) n = 6	Viability, DNA methylation (NFKB and DNMT activation)	2100 MHz 2 W/kg 2 h Co-exposure to Zn (concurrent)	Increased cell viability and NFKB and DNMT activation, reduced in presence of Zn.	Ergun et al. (2022)
Primary hippocampal embryonic rat neurons (PHNs) n = 16-48 cells	Neuronal excitability, intracellular calcium, synaptic transmission	3 GHz 0.3 W/kg 1 h	Decreased eAP amplitude; resting membrane potential (MP) depolarization; increased AP duration, basal Ca^{++} level and synaptic activity. No effects on plasma membrane (PM) resistance and cell proliferation.	Echchgadda et al. (2022)
Rat adrenal pheochromocytoma cells (PC12) n = 3	autophagy	2856 MHz 19 W/kg 15 min	Activation of autophagy, measured as LC3-II increase (restored at 12 h post-exposure), increased expression of Beclin1, upregulation of Atg5, 7 and 9, increased number of autophagosomes and autolysosomes, down-regulation of miR-30a and increased expression of AMPKα2.	Hao et al. (2022)
Human neuroblastoma cells (SH-SY5Y) n = 3-4	DNA damage and autophagy	1950 MHz, UMTS 0.3 W/kg 20 h Co-exposure to MD (after RF)	No effect of RF alone on DNA damage. Reduction of MD-induced DNA damage by pre-exposure. Negation of RF-induced protective effects in cells chemically and genetically inhibited for autophagy.	Sannino et al. (2022)
Human neuroblastoma cells (SH-SY5Y) Colon cancer cells (HCT116) Cultures of primary cells from rat cortex (astrocytes and co-cultures of neurons and glial cells) n = 4 to 11	Global cellular response	1800 MHz, GSM, UMTS, LTE, Wi-Fi, CW 1.5 – 24 W/kg, 3 – 5 days	No effect.	Joushomme et al. (2022)

Abbreviations: AMPKα2: Adenosine monophosphate-activated protein kinase-α2; AP: Action potential; Atg: autophagic genes; Ca++: Calcium ions; CW: Continuous wave; DNMT: DNA methyltransferase; eAP: evoked action potential; GSM: Global System for Mobile Communications; LTE: Long-Term Evolution; MD: menadione; MP: membrane potential; NFKB: Nuclear factor kappa; PM: plasma membrane; UMTS: Universal Mobile Telecommunications System; Wi-Fi: Wireless Fidelity; Zn: Zinc.

4.4.6 Conclusions on RF cell studies

As in previous years, there is a large variety of endpoints and cell types investigated with varying results. The exposure parameters, such as frequency (from 915 MHz to 3 GHz), modulation (CW, GSM, UMTS, LTE, WiFi), exposure duration (from few minutes to 60 days), and exposure level (from 0.0008 to 24 W/kg) also vary significantly among studies. Therefore, it is difficult to draw general conclusions.

In addition, it must be stressed that, among the studies recognized, more than 60% (13 of 20) have not been considered due to the low experimental quality (mainly lack of dosimetry and/or sham-controls), as reported in the appendix.

Excluded Studies

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

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

1.b) Papers that did not study any health outcome and/or biological effect (including letters, commentaries etc.).

1.c) Papers that did not in any way study the association between radiofrequency fields and a health outcome and/or a biological effect (e.g., the use of text messages for self-management of diabetes).

1.d) Studies on using EMF as therapeutic interventions (e.g. diathermy, osteoporosis, bone healing, diabetes, schizophrenia, spinal cord injury,…).

1.e) Case-reports.

1.f) Not a peer-reviewed publication, or published in another language than English.

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

1.h) Narrative reviews.

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

1.j) Insufficient or missing exposure description and/or dosimetry in human, animal and in vitro studies. This includes studies addressing exclusively exposure assessment methods which have been proven to be invalid such as self-estimated distance to mobile phone base stations.

2.a) Studies that did not include humans. Note that studies of humans with an experimental design are included in the chapter “human studies”.

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

2.c) 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.

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

2.e) Statistics not described and/or confounders not adjusted for.

Epidemiological Studies

Almekhlafi et al. [123]	1.b
Agence national des fréquences [124]	1.f
Arribas et al. [125]	1.b
Barbosa et al. [126]	1.b
Beaudin et al. [127]	1.a
Belpomme et al. [128]	1.h
Bhatt et al. [129]	1.b
Biering et al. [130]	1.a
Boussad et al. [131]	1.b
Calderón et al. [132]	1.b
Cerezci et al. [133]	1.b
Colella et al. [134]	1.b
Costantino et al. [135]	1.b
De Vocht et al. [136]	1.b
Deprez et al. [137]	1.b
Dieudonné et al. [138]	1.b
Duris et al. [139]	1.b
Gallucci et al. [140]	1.b
Garcia-Cobos et al. [141]	1.b
Ghanbari et al. [142]	1.b
Gocsei et al. [143]	1.b
Grochans et al. [144]	1.h
Gryz et al. [145]	1.b
Hamiti et al. [146]	1.b
Hardell et al. [147]	1.e
Hardell et al. [148]	1.f
Ikuyo et al. [149]	1.b
Jalilian et al. [65]	1.b
Jangid et al. [150]	1.h
Jeschke et al. [151]	1.b
Kapetanakis et al. [152]	1.b
Karpat et al. [153]	1.b
Kitajima et al. [154]	1.b
Koppel et al. [155]	1.b
Koppel et al. [156]	1.b
Lemay et al. [157]	1.b
Leszczynski et al. [158]	1.h
Lin et al. [159]	1.h
Maffei et al. [160]	1.h
Markussen et al. [161]	1.b
Mezei et al. [162]	1.d
Mijatovic et al. [163]	1.b
Mohammed et al. [164]	1.h
Moskowitz et al. [165]	1.b
Najera et al. [166]	1.b
Nielsen et al. [167]	1.a
Onyije et al. [168]	1.h
Pradhan et al. [169]	1.b
Schmutz et al. [170]	1.b
Schüz et al. [56]	1.f
SwissNIS [171]	1.f
de Vocht et al. [172]	1.h
Yamaguchi-Sekino et al. [173]	1.b
Yamaguchi-Sekino et al. [174]	No blinding
Yamazaki et al. [175]	1.b
Yang et al. [176]	3.b
Zhang et al. [177]	1.b

Human studies

ELF

Exposure	Reference	Reason for exclusion
PEMF	Ong et al. [178]	1.d)
PEMF	Perumal et al. [179]	1.d)
PEMF	Tong et al. [180]	1.d)
PEMF	Wagner et al. [181]	1.d) 1.e)
ELF	Shinba et al. [182]	3.d) (no information on blinding of subjects, experimenter was not blind)
ELF	Skomro et al. [183]	1.d)

RF

RF	Díaz-Del Cerro et al. [184]	3.a), 3.d)
RF	Dömötör et al. [185]	1.e), 3.c)
RF	Dömötör et al. [186]	1.e), 3.c)
RF	Chen et al. [187]	1.d)
RF	Nayak et al. [188]	1.j), 3.c)
RF	Pattnaik et al. [189]	1.j)
RF	Schneider et al. [190]	3a), 3.d)
RF	von Klitzing L. [191]	1.e), 3.c)

Animal Studies

Static

Reference	Reason for exclusion
Bianco et al. [192]	Geomagnetic field orientation
Brito et al. [193]	Therapeutic effect of EMF (1.d)
Collett et al. [194]	Geomagnetic field orientation
Feng et al. [195]	Therapeutic use of EMF (1.d)
Fleischmann et al. [196]	Geomagnetic field orientation
Guerra et al. [197]	Geomagnetic field orientation
Heyers et al. [198]	Geomagnetic field orientation
Jandačka et al. [199]	Geomagnetic field orientation
Karwinkel et al. [200]	Magnetoreception study
Karwinkel et al. [201]	Magnetoreception study
Krylov et al. [202]	Study used earth geomagnetic variations
Lv et al. [203]	Therapeutic effect of EMF (1.d)
Pakhomov et al. [204]	Geomagnetic field orientation
Song et al. [205]	Description of sham group absent (2.c)
Tong et al. [206]	Therapeutic use of EMF, effects on microbial (1.d)
Wang et al. [207]	Therapeutic use of EMF (1.d)
Wynn et al. [208]	Geomagnetic field orientation
Yu et al. [209]	Therapeutic use of EMF (1.d)
Zhan et al. [210]	Geomagnetic field orientation
Zhang et al. [211]	Experimental animal number not reperted

ELF

Reference	Reason for exclusion
Ambalayam et al. [212]	Therapeutic use of EMF (1.d)
Azizi et al. [213]	Therapeutic use of EMF, Dosimetry (1.d) (1.j)
Chakraborty et al. [214]	Therapeutic use of EMF (1.d)
Harakawa et al. [215]	Therapeutic use of EMF (1.d)
Harakawa et al. [216]	Therapeutic use of EMF (1.d)
Huang et al. [217]	Therapeutic use of EMF (1.d)
Kantar et al. [218]	Therapeutic use of EMF (1.d)
Khan et al. [219]	Therapeutic use of EMF (1.d)
Tekutskaya et al. [220]	No ethical clearence reported, no dosimetry (1.j)
Tony et al. [221]	No sham, insufficient dosimetry (1.j, 2.c)

RF

Paper	Reason for exclusion
Young et al. [222]	Description of study design.
Farah et al. [223]	No dosimetry. (1.j)
Noha et al. [224]	No dosimetry. (1.j)
Wei-Jia et al. [225]	Paper in Chinese. (1.f)
Zhang et al. [226]	Paper in Chinese. (1.f)
Abdalla et al. [227]	No dosimetry. (1.j)
Ayla et al. [228]	No sham-exposed group. (2.c)
Yucel et al. [229]	No sham-exposed group. (2.c)
Imam et al. [230]	No sham-exposed group. (2.c)
Orhan et al. [231]	No sham-exposed group. (2.c)
Fatma et al. [232]	No sham-exposed group. (2.c)
Pagadala et al. [233]	No dosimetry.(1.j)
Van Nhut Khanh et al. [234]	No sham-exposed group, no dosimetry. (2.c)(1.j)
Jafari et al. [235]	No dosimetry. (1.j)
Dayan et al. [236]	Most likely thermal effects.
Galal et al. [237]	No dosimetry. (1.j)
Dsilva et al. [238]	No dosimetry. (1.j)
Khodamoradi et al. [239]	No sham-exposed group, incomplete dosimetry. (2.c)(1.j)
Borzoueisileh et al. [240]	No sham-exposed group, incomplete description of exposure setup. (2.c)(1.j)
Zhao et al. [241]	Incomplete dosimetry (no exposure time). (2.c)(1.j)
Singh et al. [242]	4.e) N<5
Singh et al. [243]	4.e) N<5
Zhu et al. [244]	4.e) N<5
Pardhiya et al. [245]	4.e) N<5
Martinelli et al. [246]	1.j) Incomplete dosimetry (no exposure level). (1.j)
Ustunova et al. [247]	No sham-exposed group. (2.c)

Cell Studies

Static

Reference	Reason for exclusion
Synowiec-Wojtarowicz et al. [248]	number of independent experiments < 3

ELF

Reference	Reason for exclusion
Maleki et al. [249]	number of independent experiments not reported

INT

Reference	Reason for exclusion
Kim et al. [250]	No dosimetry performed; no Sham control (1.j, 2.c)

RF

Reference	Reason for exclusion
Bertagna et al. [19]	Cell phone in on mode; no dosimetry performed (1.j)
Ranjitsingh, A. J. A., et al. [251]	Cell cultures on a laptop; no dosimetry performed (1.j)
Lawler et al. [252]	No Sham control (2.c)
Costantini et al. [253]	Medical device used for exposure. No dosimetry performed (1.j)
Gökçen, S., et al. [254]	No dosimetry performed (1.j)
Kim et al. [255]	No Sham control (2.c)
Hassanzadeh-Taheri et al. [256]	Cell phone in on mode; no Sham control (1.j, 2.c)
Yin et al. [257]	No dosimetry performed; no Sham control (1.j, 2.c)
Olejárová et al. [258]	Sham-control carried out covering cell cultures with a “radiofrequency protective foil” (1.j)
Ploskonos et al. [259]	Medical device used for exposure; no Sham control (1.j, 2.c)
Senturk et al. [260]	No dosimetry performed; no Sham control (1.j, 2.c)
Tayebi et al. [261]	No Sham control (2.c)
Pooam et al. [262]	No dosimetry performed; no Sham control (1.j, 2.c)
Martinelli et al. [246]	No dosimetry. (1.j)

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