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# NTP Turns to Mechanisms

DNA Breaks, Oxidative Stress and  
Gene Expression Are on the Agenda

**September 17, 2019**

**Last updated**

**October 22, 2019**

The U.S. National Toxicology Program ([NTP](https://ntp.niehs.nih.gov/)) will soon embark on a new phase of its long-running RF project. Last year, the NTP [concluded](https://microwavenews.com/news-center/ntp-final-rf-report) that RF radiation causes cancer; now it will begin a systematic search for mechanisms to explain how and why the tumors developed. Work is expected to begin by the end of the year.

The research plan is wide-ranging. It includes studies on gene expression, oxidative stress and DNA damage and repair, as well as the possible role played by heat. Other priorities on the NTP agenda are studies on behavior and stress.

We’re “optimistic” that we can detect changes in gene expression and identify biomarkers of RF effects, NTP’s [Michael Wyde](https://www.niehs.nih.gov/research/atniehs/labs/tob/systems/staff/wyde/index.cfm) told Microwave News. Wyde is leading the new project. He will continue to work with [John Bucher](https://irp.nih.gov/pi/john-bucher), the former NTP associate director, who ran its $30 million animal study, which showed [“clear evidence”](https://microwavenews.com/news-center/ntp-final-rf-report) that RF radiation can lead to malignant tumors in male rats.

The NTP has already [reported](https://microwavenews.com/news-center/ntp-comet-assay) finding more DNA breaks —as detected with the comet assay— among the RF–exposed animals, including in the brain where rats later developed tumors. Those results, [presented](https://twitter.com/MicrowaveNews/status/910170210010849281) at a conference two years ago, have been submitted for publication. The paper is currently under peer review, according to [Sheena Scruggs](https://www.linkedin.com/in/sheenafaherty) in [NIEHS](https://www.niehs.nih.gov/)’ Office of Communications and Public Information. (The NTP and NIEHS are closely connected.)

**How Does RF Radiation Cause DNA Breaks?**

The fact that the NTP documented DNA damage “adds to the credibility of the animal findings,” said [Ron Melnick](https://www.youtube.com/watch?v=nJfK3gbkmMk). “It’s very supportive.” Melnick led the team that designed the NTP study; he retired in 2009.

Still missing, however, is how RF radiation causes DNA damage. “The breaks themselves don’t tell you anything about the mechanism at work,” [Henry Lai](https://bioe.uw.edu/portfolio-items/henry-lai/) explained in a recent interview. Twenty-five years ago, Lai and [N.P. Singh](https://microwavenews.com/news-center/singh-comet-assay-radiation-research) were the first to [show](https://onlinelibrary.wiley.com/doi/abs/10.1002/bem.2250160309) that RF radiation can induce DNA breaks —as it happened, in the brains of rats.

It is generally accepted that RF radiation is in itself not powerful enough to break chemical bonds and therefore unable to directly tear DNA apart. At the outset, Lai and Singh offered two possible mechanisms: oxidative stress and impaired DNA repair. Oxidative stress is shorthand for the sequence of events that follows an increase in the number of free radicals —biologically active molecules that can damage DNA. Alternatively, RF radiation may hinder the cell’s ability to repair DNA breaks, which occur naturally and not infrequently.

In 1997, two years after their original [paper](https://onlinelibrary.wiley.com/doi/abs/10.1002/bem.2250160309), Lai and Singh [followed up](https://onlinelibrary.wiley.com/doi/abs/10.1002/(SICI)1521-186X(1997)18:6%3C446::AID-BEM7%3E3.0.CO;2-2) with strong evidence implicating oxidative stress. When they treated the rats with melatonin —a natural hormone that neutralizes free radicals— before RF exposure there were no more DNA breaks. If the radiation could indeed generate free radicals, they pointed out, the risks would go beyond cancer to include premature aging as well as Alzheimer’s, ALS and other neurological diseases.

“If I were to design the project, I would look at the link between oxidative stress and DNA damage,” Melnick said. “That’s doable.”

A recent [review](https://www.tandfonline.com/doi/abs/10.3109/15368378.2015.1043557) of some 100 journal articles found that more than 90 percent “confirmed that [low-level] RF radiation induces oxidative effects in biological systems.” It was published in Electromagnetic Biology and Medicine in 2016.

NTP’s Wyde said that an important first step will be “to replicate the comet assays” to confirm that RF radiation damages DNA. He cited some uncertainty due to the wide variation in the extent of the breaks seen in the original NTP experiments and the small number of animals used. If the breaks are replicated, Wyde plans to run additional “more specific and robust assays” to evaluate the DNA damage and repair enzymes.

**New Smaller Exposure Chambers**

For this new phase of the RF project, the NTP has again turned to the [IT’IS Foundation](https://itis.swiss/news-events/news/latest-news/) in Zurich to design and build new [reverberation chambers](https://microwavenews.com/news-center/ntp-peer-review-sees-tumor-risk#ITIS), which are more compact and less expensive than the room-size units built for the original study. As before, these smaller units will also allow animals to move freely while being exposed to 900 MHz or 1800 MHz radiation. Each can house up to ten animals.

The NTP declined to discuss the new exposure setups, stating only that the information would be posted on the NTP [RF website](https://ntp.niehs.nih.gov/results/areas/cellphones/index.html) in due course. [Niels Kuster](https://itis.swiss/who-we-are/staff-members/all-staff/niels-kuster/), the director of IT’IS, confirmed that four new chambers have been delivered to the NIEHS/NTP Campus in Research Triangle Park, NC.

For the time being, the NTP is planning only animal studies. When asked whether in vitro RF experiments (using living cells) are under consideration, the NTP communications office replied that their feasibility is “still being assessed.”

In a recent [posting](https://ntp.niehs.nih.gov/results/areas/cellphones/index.html) on its website, the NTP announced that it is in the midst of evaluating the literature on the higher frequencies used in 5G.

**October 22, 2019**

The NTP paper on DNA breaks has been published in Environmental and Molecular Mutagenesis. The abstract is [here](https://onlinelibrary.wiley.com/doi/abs/10.1002/em.22343).

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<https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343>

[Environmental and Molecular Mutagenesis](https://onlinelibrary.wiley.com/journal/10982280)

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Research Article

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# Evaluation of the genotoxicity of cell phone radiofrequency radiation in male and female rats and mice following subchronic exposure

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First published: 21 October 2019

[**https://doi.org/10.1002/em.22343**](https://doi.org/10.1002/em.22343)

Citations: [14](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#citedby-section)

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## Abstract

The National Toxicology Program tested two common radiofrequency radiation (RFR) modulations emitted by cellular telephones in a 2‐year rodent cancer bioassay that included interim assessments of additional animals for genotoxicity endpoints. Male and female Hsd:Sprague Dawley SD rats and B6C3F1/N mice were exposed from Gestation day 5 or Postnatal day 35, respectively, to code division multiple access (CDMA) or global system for mobile modulations over 18 hr/day, at 10‐min intervals, in reverberation chambers at specific absorption rates of 1.5, 3, or 6 W/kg (rats, 900 MHz) or 2.5, 5, or 10 W/kg (mice, 1,900 MHz). After 19 (rats) or 14 (mice) weeks of exposure, animals were examined for evidence of RFR‐associated genotoxicity using two different measures. Using the alkaline (pH > 13) comet assay, DNA damage was assessed in cells from three brain regions, liver cells, and peripheral blood leukocytes; using the micronucleus assay, chromosomal damage was assessed in immature and mature peripheral blood erythrocytes. Results of the comet assay showed significant increases in DNA damage in the frontal cortex of male mice (both modulations), leukocytes of female mice (CDMA only), and hippocampus of male rats (CDMA only). Increases in DNA damage judged to be equivocal were observed in several other tissues of rats and mice. No significant increases in micronucleated red blood cells were observed in rats or mice. In conclusion, these results suggest that exposure to RFR is associated with an increase in DNA damage. Environ. Mol. Mutagen. 61:276–290, 2020. © 2019 Wiley Periodicals, Inc.

## INTRODUCTION

Over the past two decades, cellular telephone use has become nearly ubiquitous worldwide; cell phone subscriptions numbered ~7.68 billion in 2017 according to the International Telecommunication Union ([**2017**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0013)) with ~5.12 billion unique subscribers (GSMA Intelligence [**2019**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0007)). Radiofrequency radiation (RFR) is a form of electromagnetic radiation that ranges from 3 kHz to 300 GHz. Most cell phones transmit RFR signals within the 800–900 and 1,800–2,200 MHz ranges (International Agency for Research on Cancer [IARC] Working Group on the Evaluation of Carcinogenic Risks to Humans [**2013**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0012)).

Concern exists as to whether cell phone RFR frequencies are capable of adversely affecting human health. Although some epidemiological studies suggest that cell phone use might increase the risk for certain brain cancers, such as gliomas and acoustic neuromas (a,k,a, vestibular schwannomas), the odds ratios for these increased risks are quite low (INTERPHONE Study Group [**2010**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0014); Cardis et al. [**2011**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0005); Hardell et al. [**2011**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0009); Larjavaara et al. [**2011**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0016); Sato et al. [**2011**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0025); Hardell and Carlberg [**2015**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0008)). Conclusions drawn from these observations may be premature, as cell phone use has become commonplace only within the past two decades, a period of time that may be insufficient to accurately assess cancer‐related outcomes. Results of previous rodent cancer studies conducted with a variety of RFR exposures and durations are inconsistent and inconclusive, and many of these studies used experimental protocols with important limitations, indicating a need for a more definitive study (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans [**2013**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0012)).

Additionally, extensive reviews of the literature on the genotoxicity of various frequencies and modulations of RFR have concluded that evidence for RFR‐associated genotoxicity is inconsistent and weak (Brusick et al. [**1998**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0003); Ruediger [**2009**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0023); Verschaeve et al. [**2010**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0028)), and some key studies reporting RFR‐associated genotoxicity in human cell lines could not be replicated (Speit et al. [**2013**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0027)). As with the cancer studies, interpretations of the genotoxicity studies, particularly those performed *in vivo*, have also been limited by issues of experimental design. In 2013, after reviewing the available data, the IARC classified radiofrequency electromagnetic fields (RF‐EMF), which include the RFR wavelength range, as “possibly carcinogenic to humans (Group 2B),” based on limited evidence in experimental animals and limited evidence in humans on the association between RF‐EMF and cancer (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans [**2013**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0012)).

To help inform human health risk assessments, the National Toxicology Program (NTP) designed and conducted a 2‐year rodent cancer study of cell phone RFR, using code division multiple access (CDMA) or global system for mobile (GSM) modulations, the principal modulations used in the United States (CDMA and GSM) and in the rest of the world (GSM). GSM and CDMA are second‐generation (2G) and third‐generation (3G) technologies, respectively, and they differ in the method in which information is incorporated and transmitted within frequency bands. The previous inconsistent genotoxicity and tumorigenicity findings that have been reported following RFR exposure could be due in part to the immense and unique technical challenges inherent in studying the effects of nonionizing radiation, including RFR (Capstick et al. [**2017**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0004); Gong et al. [**2017**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0006)). To address these challenges and provide data to clarify possible adverse biological effects of cell phone RFR exposure, the NTP took into account numerous variables and parameters in designing its rodent cancer bioassay. Key features included construction of custom‐designed reverberation chambers that exposed animals to a clearly defined, statistically homogenous radiofrequency field, that shielded animals from all other sources of RFR, and eliminated the need for restraint, a method commonly employed by other researchers for point‐source exposures (Capstick et al. [**2017**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0004); Gong et al. [**2017**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0006)). Animals were housed inside the reverberation chambers and exposed to RFR for a total of 9 hr 10 min per day in 10‐min on/off cycles (over the course of an ~18 hr period) at frequencies with modulations being used in cellular networks (Capstick et al. [**2017**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0004)). In addition, the exposure levels selected for this study were based on the results of previously conducted dosimetry studies and thermal pilot studies that demonstrated no measurable hyperthermia in rats and mice at the exposure levels chosen for this study (Gong et al. [**2017**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0006); Wyde et al. [**2018**](https://onlinelibrary.wiley.com/doi/full/10.1002/em.22343#em22343-bib-0030)).

In the NTP study design, Sprague Dawley rats and B6C3F1/N mice of both sexes were whole‐body exposed to RFR (CDMA or GSM modulations). Rats were exposed *in utero* beginning on Gestation day 5 (GD5), and mice were exposed beginning at 5 weeks of age. After a total of 19 weeks of exposure for rats and 14 weeks for mice, subsets of 5 rats and 5 mice of each sex from each exposure group were removed from the ongoing 2‐year cancer bioassay after subchronic exposure and assessed for DNA damage using the comet assay, and for changes in chromosomal structure and/or number using the peripheral blood erythrocyte micronuclei (MN) assay. For the comet assay, cells from three functionally distinct structures of the brain (frontal cortex, hippocampus, and cerebellum), along with liver cells and peripheral blood leukocytes were assessed. Brain tissue was analyzed in the comet assay due to concerns that RFR may increase risk for brain cancer in humans, whereas liver cells and blood leukocytes were selected for analysis as these cells are part of typical analyses conducted at the NTP for DNA damage.

(go to this website to read entire article)