# Evaluation of the Genotoxicity of Cell Phone Radiofrequency Radiation in Male and Female Rats and Mice Following Subchronic Exposure

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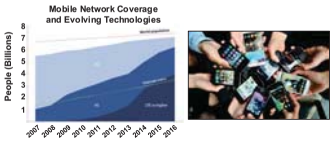
## Abstract

The National Toxicology Program tested the two common radiofrequency radiation (RFR) modulations emitted by cellular telephones in a 2-year rodent cancer bioassay that included additional animal cohorts for interim assessments of genotoxicity endpoints. Male and female Sprague Dawley 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 (GSM) modulations semi-continuously for 18 h/day in 10 min intervals in reverberation chambers at specific absorption rates (SAR) of 1.5, 3, or 6 W/kg (rats) or 2.5, 5, or 10 W/kg (mice). Rats and mice were exposed at 900 MHz or 1900 MHz, respectively. The interim cohorts, 5 animals per treatment group, were examined after 19 (rats) or 13 (mice) weeks of exposure for evidence of RFR-induced genotoxicity. DNA damage was assessed in three brain regions (frontal cortex, hippocampus, and cerebellum), and in liver cells and blood leukocytes using the comet assay. Chromosomal damage was assessed in peripheral blood erythrocytes using the micronucleus assay. DNA damage was significantly increased in the frontal cortex of male mice (both modulations), peripheral leukocytes of female mice (CDMA only), and hippocampus of male rats (CDMA only). DNA damage was nominally elevated in several other tissues of RFR-exposed rats, although statistical significance was not achieved. No significant increases in micronucleated red blood cells were observed in rats or mice. These results suggest that exposure to RFR has the potential to induce measurable DNA damage under certain exposure conditions.

## Introduction

- Cellular telephone use is nearly ubiquitous world-wide: cell phone subscriptions were estimated at 6.9 billion in 2014.






- Cell phones transmit radiofrequency radiation (RFR) signals; RFR is a form of electromagnetic radiation.
- Whether exposure to RFR via cell phones can cause cancer, particularly brain cancer, in humans has been of concern. IARC classified radiofrequency electromagnetic fields (RF-EMF), as "possibly carcinogenic to humans (Group 2B)," based on limited evidence in experimental animals and insufficient evidence in humans to support a conclusion on the association between RF-EMF and cancer.
- Results of previous rodent cancer and genotoxicity studies of varying RFR exposures and durations are inconsistent and inconclusive, and many of these studies used experimental protocols with significant limitations. Hence, there is still much uncertainty about the possible adverse effects of RFR, as reflected by the IARC classification.
- The Food and Drug Administration (FDA) Center for Device and Radiation Health nominated Radiofrequency Radiation Emissions of Wireless Communication Devices to the NTP as a high priority nomination in 1999.
- To help inform human health risk assessments, the NTP conducted a 2-year rodent cancer bioassay of the modulations of RFR most commonly emitted by cell phones.
- Genotoxicity testing was conducted using subsets of rats and mice exposed under the same experimental design as the cancer bioassay, albeit for shorter durations.

## Study Design, Materials & Methods

**Study Design**

Male and Female Sprague Dawley Rats (5 rats per exposure group)
- 19 weeks of exposure beginning ~gestational day 5
- 1.5, 3.0, or 6.0 W/kg CDMA or GSM (900 MHz)
- One sham control for each sex

Male and Female B6C3F1/N Mice (5 mice per exposure group)
- 13 weeks of exposure beginning ~postnatal day 35
- 2.5, 5.0, or 10.0 W/kg GSM or CDMA (1900 MHz)
- One sham control for each sex

**Whole Body Exposure**

**Please see Capstick et al. (2017) and Gong et al. (2017) for extensive details**

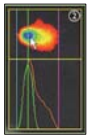
- Daily from 11:00 AM to 2:00 PM and 3:40 PM to 7:00 AM
- RFR cycled on and off every 10 min during exposure periods
  - Total duration of exposure of 9 h 10 min per 24 h period
- An upper limit of 1 °C (1.8 °F) was set as an acceptable increase in body temperature. In 5- and 28-day pilot studies, significant increases in body temperature were rare in rats and mice exposed to 6 or 10 W/kg, respectively (either modulation), and such increases, when they occurred, were < 1 °C. Body temperatue increases > 1 °C were expected to be highly unlikely in this study (Wyde et al., submitted)

**RFR Exposure Facility at Illinois Industrial Research Institute (ITRI)**

- Reverberation chambers and animal housing were developed in collaboration with the National Institute of Standards and Technology (NIST) and the Foundation for Research on Information Technologies in Society (IT'IS).
- Reverberation chambers created uniform fields of RFR and shielded animals from all other sources of RFR.
- Field uniformity was achieved by installing excitation antennas with rotating horizontal and vertical reflective surface paddles to ensure even distribution of statistically homogenous RFR fields.
- Cages, cage racks, and materials used to deliver food and water were designed to minimize interference with RFR exposure; e.g., specialized lixits were developed to prevent drinking tubes from acting as antennas for RFR.
- RFR field intensity, uniformity, quality of modulation, and numerous other parameters were validated by NIST.
- Consistency of exposure was monitored in real time by IT'IS.

**Comet Assay**

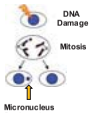
Frontal cortex, hippocampus, cerebellum, liver, and peripheral blood were analyzed in the comet assay. Single-cell suspensions were diluted in agarose and layered onto CometSlides™. Slides were incubated overnight in lysing solution at 4 °C, then treated with cold alkali solution for 20 min to allow DNA unwinding. Slides were then subjected to electrophoresis for 20 min. After staining with SYBR® Gold, slides were coded to mask treatment and scored using Comet Assay IV Imaging Software. DNA migration was measured in 100 non-overlapping comet images per animal/tissue and reported as % Tail DNA. Hedgehogs (HH; all DNA appears by visual inspection to be in the tail) were scored as a separate category.



Comet Assay IV imaging software identifies the left edge of the head of the comet and flips it to construct the right edge. % Tail DNA is calculated by dividing the fluorescence intensity of the tail by the total fluorescence intensity of the entire comet figure.

**Micronucleus Assay**

Flow cytometric analysis was performed using MicroFlow$^{PLUS}$ Kit reagents and a FACSCalibur™ system. Reticulocytes (RET) and mature erythrocytes (E) were analyzed for micronuclei (MN). For each sample, ~20,000 RET were analyzed and ~1 x $10^6$ mature erythrocytes were enumerated concurrently, allowing for calculation of the %RET among total erythrocytes as a measure of bone marrow toxicity. The protocol was consistent with OECD Guideline 474.




Micronucleus (broken chromosomes or whole chromosome loss)

Results for MN-RETs, MN-Es, and %PCEs were negative for both species, both sexes, and both RFR modulations (data not shown).

## Figure 1

**Two Approaches for Scoring Comets**

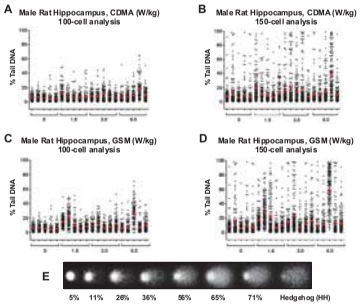



**Fig. 1A, C**: Comets were selected by a scorer (blind to treatment) for analysis *via* software to determine % Tail DNA. 100 cells were analyzed per animal/tissue and HH – identified by visual inspection – were tabulated but excluded from analysis. However, using this approach, % Tail DNA rarely exceeded 65%, yet for some tissues %HH values were markedly elevated. **Fig. 1B, D**: OECD TG 489 (OECD, 2014) recommends analyzing 150 cells. To be consistent with this new recommendation, and due to inter-animal variability observed in rats, rat slides were re-analyzed using this method; all scorable cells were analyzed with imaging software (i.e., visual inspection alone was not used to eliminate HH). This approach revealed a broader spectrum of DNA damage (Fig. 1B & D). There were few changes in statistically significant results based on scoring 100 vs. 150 cells. **Fig. 1E**: Representative images of DNA migration in the comet assay (% Tail DNA) from male rat frontal cortex.

## Figure 2

**Positive Results**

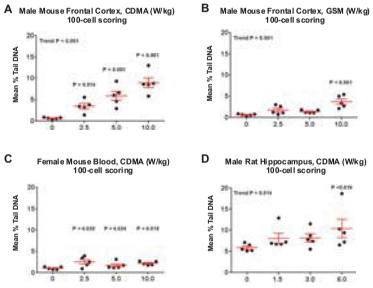
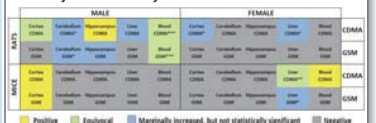



**Fig. 2A-D**: Of the 40 tissues examined (5 tissues, 2 species, 2 sexes, 2 modulations) using the 100-cell scoring approach, 4 showed positive results using the following criteria: significant trend test ($P < 0.025$) and at least one significant dose group ($P < 0.025$); or at least 2 significant dose groups. Similar results were obtained for these tissues when data were analyzed using the 150-cell method except for male rat hippocampus (means of all exposed groups were greater than the control, but did not reach statistical significance).

Tissues from rats tended to show greater inter-animal variability than those from mice. This inter-animal variability may reflect the genetic diversity of this outbred rat stock. However, % Tail DNA values from different tissues from the same rat rarely correlated, suggesting inter-tissue variability as well.

## Figure 3

**Summary of Comet Assay Results**




**Positive**: Significant trend test ($P < 0.025$) and at least one significant dose group ($P < 0.025$); or at least 2 significant dose groups. **Equivocal**: Either a significant trend test or one significant dose group. **Marginally increased**: Means of all exposed groups were higher than the control and P values approached statistical significance. **Negative**: none of the above.

In summary, 8/40 tissues exhibited some degree of effect from RFR exposure when assessed using the 100-cell scoring approach. Five tissues exhibited marginal, but statistically non-significant increases when assessed using the 150-cell scoring approach (*). Female mouse liver (CDMA) went from marginal to an equivocal call using the 150-cell scoring approach (**). Two tissues, male rat blood (CDMA and GSM), went from negative to equivocal calls using the 150-cell scoring approach (***).

## Conclusions

- When considering both scoring methods, 15/40 of the tissues examined showed some indication of response (positive, equivocal, or increases in % Tail DNA in the absence of statistical significance) in the comet assay. The comet assay is a hazard identification assay, and the damage detected by the assay represents a snapshot of the kinetics of DNA damage and repair processes.
- In the 2-year bioassay, a low incidence of malignant gliomas of the brain was observed in male, but not female, rats exposed to CDMA or GSM in the 2-year cancer bioassay (Wyde et al. 2016). Results are not yet available for mice. Considering that male rat brain tissue was more affected by RFR in the comet assay compared to female rats and male and female mice, it will be of interest to see whether there is a correlation between the comet assay results and the complete findings from the cancer bioassay.
- High exposure levels of RFR can cause hyperthermia in rats and mice, and hyperthermia is known to cause genotoxic effects in both the comet and micronucleus assays; however, the exposures used in the 2-year cancer bioassay (and therefore the genetic toxicity studies) were carefully selected, based on pilot study data, to avoid thermal effects.
- The mechanism by which RFR could induce biological effects other than by increasing body temperature is a matter of intense speculation. The NTP is currently in the process of acquiring smaller RFR whole body exposure chambers for follow-up studies. Initial studies will aim to explore potential mechanisms underlying the observed DNA damage in the comet assay and explore other biomarkers of genetic damage.

## References

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