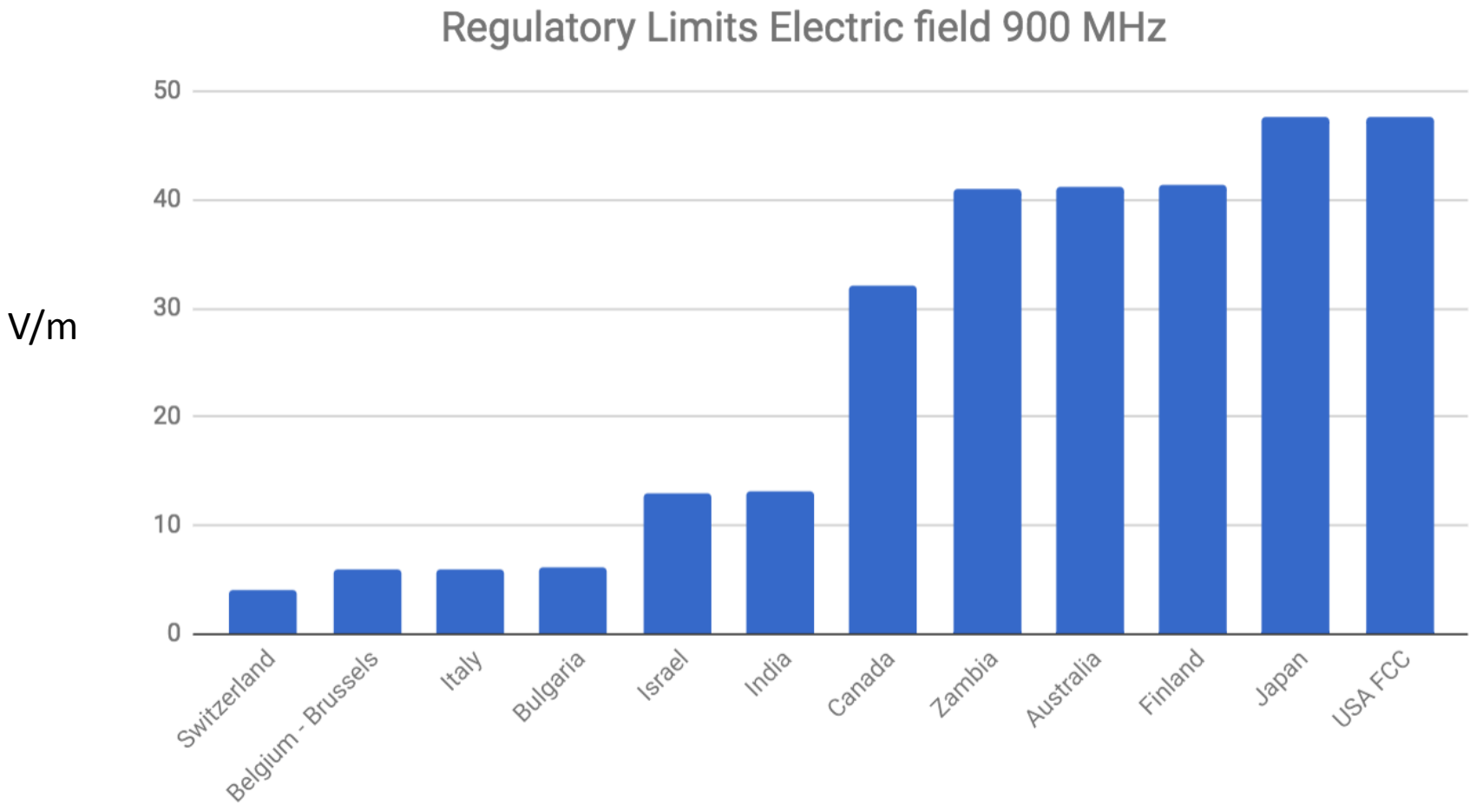
Comments on the National Toxicology Program Study of Radiofrequency Radiation Technical Report
March 26, 2018

Theodora Scarato


Regulatory Limits Electric field 900 MHz
V/m
50
40
30
20
10
0
Switzerland
Belgium - Brussels
Italy
Bulgaria
Israel
India
Canada
Zambia
Australia
Finland
Japan
USA FCC

Cell Phone Calls and ADHD
Potentiation of Lead Toxicity

ADHD symptoms associated with voice calls, *only among children with higher blood lead values. (Byun 2013 PLOS)*

> "Our results suggest that exposure to RF associates with increased ADHD symptom risk with simultaneous exposure to lead, and that RF exposure alone may have a weak or no effect on ADHD symptoms, i.e., a combined or cooperative toxic action of RF and lead on the developing brain. "
>
> 2,422 children - 27 schools - 10 Korean cities - 2 y follow up

Increased risk of having poor/delayed neurodevelopment up to 36 months of age in association with mobile phone use during pregnancy. (Choi 2017)

- 1198 mother-child pairs, personal exposure measurements with meters and blood lead level during pregnancy.

Co-exposures represent a critical research area, significant data gaps.

Changes in the Permeability of the Blood Brain Barrier

Additional Research to be added to NTP

"This study demonstrated, for the first time, the blood-brain barrier and cognitive changes in rats exposed to 900 MHz electromagnetic field (EMF) and aims to elucidate the potential molecular pathway underlying these changes. Researchers found that EMF exposure for 28 days induced the expression of mkp-1, resulting in ERK dephosphorylation. Taken together, these results demonstrated that exposure to 900 MHz EMF radiation for 28 days can significantly impair spatial memory and damage BBB permeability in rat by activating the mkp-1/ERK pathway."

Tang, J., et al. "Exposure to 900 MHz electromagnetic fields activates the mkp-1/ERK pathway and causes blood-brain barrier damage and cognitive impairment in rats." *Brain Research*, vol. 1601, 2015, pp. 92-101.

Leszczynski, D., et al. "Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer- and blood-brain barrier-related effects." *Differentiation*, vol. 70, no. 2-3, 2002, pp. 120-9.

Sirav, Bahriye, and Nesrin Seyhan. "Effects of radiofrequency radiation exposure on blood-brain barrier permeability in male and female rats." *Electromagnetic Biology and Medicine*, 30.4 (2011): 253-260.

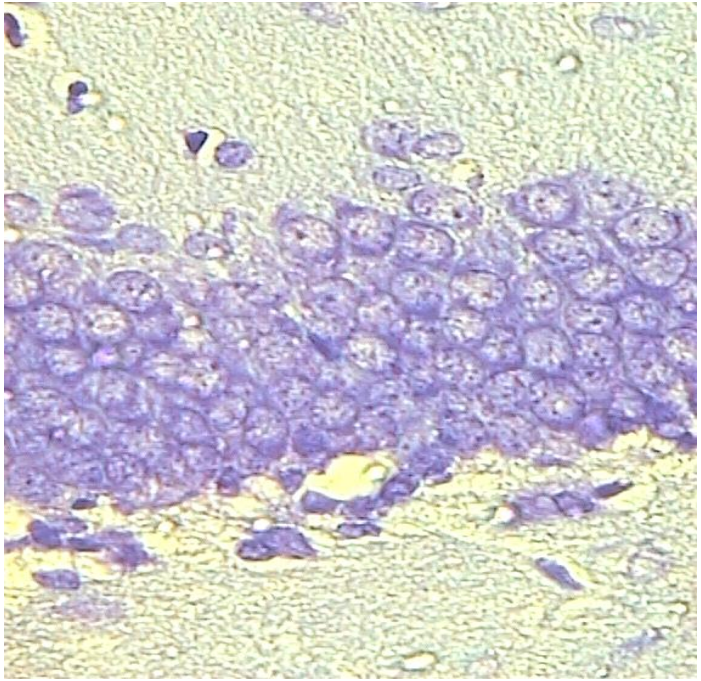
"A significant increase in albumin was found in the brains of the RF-exposed male rats when compared to sham-exposed male brains."

Sirav B, Seyhan N. "Effects of GSM modulated radio-frequency electromagnetic radiation on permeability of blood-brain barrier in male & female rats," *J Chem Neuroanat.* 2016 Sep;75(Pt B):123-7

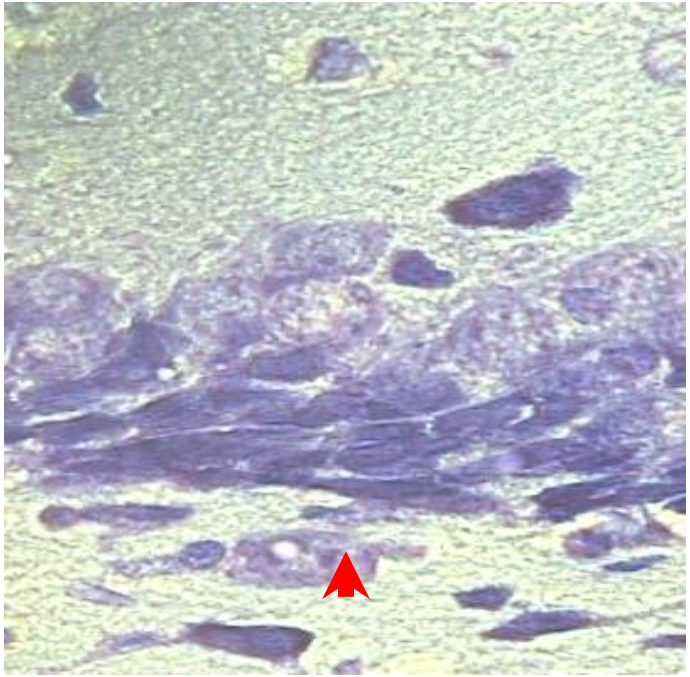
Prenatal Exposure Decreases Brain Cells

Prenatal 900 MHz EMF exposure decreased hippocampal granular cells in dentate gyrus of newborn rats.

Control Group



EMF Exposed



Odaci et al, 2009

Several studies finding decreased brain cells; Sonmez 2010, Bas 2009, Odaci 2008, Kaplan 2016

Prenatal Cell Phone Exposure Linked to Behavioral Problems Speech Issues

Mice exposed prenatally: hyperactivity and poor memory, altered brain development (Aldad 2012)

Maternal EMF exposure to speech issues in children (Zarei et al, 2015)

Prenatal/Postnatal cell phone use associated w/ behavioral problems (Divan et al., 2012 & 2009)

World Wide Government Action to Reduce RF Exposure

Over 20 countries recommend reducing exposure to cell phones

France: Cell Phone radiation labeling, no advertising cell phones to children, Wi-Fi Banned in Kindergarten. Elementary Schools Wi-Fi off when not in use.

Belgium: Ban on sale of cell phones for children. Wi-Fi banned in Ghent nursery schools.

Israel: Official recommendations to reduce exposures from phone. Wi-Fi banned in nurseries. Wi-Fi removed/ minimized in schools.

India: Phone Radiation labeling, Recommendations to reduce cell phone exposure, Exposure limits lowered to 1/10 of the ICNIRP level, some municipalities ban towers near schools.

Cyprus: Wi-Fi removed from elementary classrooms in 2017. PSAs by Cyprus Children's Environmental Health Committee

French Polynesia: Banned advertising cell phones to children under 14. Children should not use phones under 14. Major awareness campaign on how to reduce ELF/RF EMF

Maryland State Children's Environmental Health and Protection Advisory Council,

19 Members (pediatricians, public health, legislators)

"The Council recommends limiting radiofrequency exposures as much as feasibly practical."

Recommendations

The Maryland State Department of Education

"Should consider using wired devices in classrooms"

"If a new classroom is to be built... network cables can be added at the same time, providing wired network access."

"*The Maryland Department of Health and Mental Hygiene should* provide suggestions to the public on ways to reduce exposure.

San Francisco & Burlingame, California

Connecticut Department of Health,

California Department of Public Health

RECOMMENDS

SPEAKERPHONE OR HEADPHONES

SLEEP WITH PHONE AT ARM'S LENGTH

AVOID KEEPING IN POCKET

CBS THIS MORNING SATURDAY

WARNING SIGNALS

CALIFORNIA HEALTH OFFICIALS WARN ABOUT CELL PHONE SAFETY

CDPH *Deleted* Several Recommendations

144 pages of redrafts since 2009

Cordless phones : “both base stations and the handsets emit EMFs”

- “reducing time” spent on cordless phones
- “do not sleep with the base station near your head,”
- “replace old analog cordless phones with corded phones”

“What State Governments and It’s Employees Can Do To Lower Potential Risks From Cordless Phones and Cell Phones”

- “use a beeper so that cell phones can be turned off”
- “state employees could avoid purchasing cordless phones for office use.”

Ways the California State Department of General Services can take to reduce employee exposure. When purchasing phones for employees “create contract language to require manufacturers to provide SAR ratings for phones and to offer low-emission accessories.”

“Do not allow children to use a cell phone except for emergencies.”

https://www.cdph.ca.gov/Programs/OPA/Pages/NR17-086.aspx

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

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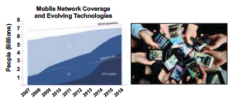
Abstract

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 temperature 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.

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.

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

Figure 1

Two Approaches for Scoring Comets

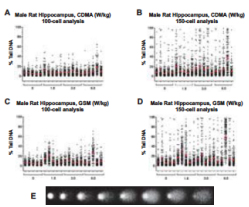


Fig. 1A, C: Comets were selected by a scorer (blind to treatment) for analysis via software to determine % Tail DNA. 100 cells were analyzed per animal/tissue and HH – identified by visual inspection – were tabulated but excluded from analysis. However, using this approach, % Tail DNA rarely exceeded 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

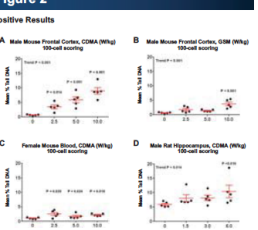
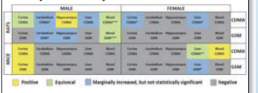


Fig. 2A-D: Of the 40 tissues examined (5 tissues, 2 species, 2 sexes, 2 modulations) using the 100-cell scoring approach, 4 showed positive results using the following criteria: significant trend test ($P < 0.025$) and at least one significant dose group ($P < 0.025$); or at least 2 significant dose 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

Capstick et al. (2017) A radio frequency radiation exposure system for rodents based on reverberation chambers. *IEEE Trans Electromagn Compat*, 59(4): 1041-1052.

Gong et al. (2017) Life-time dosimetric assessment for mice and rats exposed in reverberation chambers for the two-year NTP cancer bioassay study on cell phone radiation. *IEEE Trans Electromag Compat*, in press.

International Agency for Research on Cancer (2013) Non-ionizing radiation, Part 2: Radiofrequency electromagnetic fields. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 102(Pt 2):1-460.

Organization for Economic Cooperation and Development (2014) Test No. 474: Mammalian Erythrocyte Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

Organization for Economic Cooperation and Development (2014) Nets No. 489: In Vivo Mammalian Alkaline Comet Assay, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

Wyde et al. (2018) Report of partial findings from the National Toxicology Program carcinogenesis studies of cell phone radiofrequency radiation in Hsd: Sprague Dawley rats (whole body exposure). bioRxiv. doi: http://dx.doi.org/10.1101/059899

Wyde et al. (submitted) Design and validation of the National Toxicology Program's Cell Phone Radiofrequency Radiation Reverberation Chamber Exposure System. *Bioelectromagnetics*.

DNA Damage Analysis is Missing

"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)…"

"These results suggest that exposure to RFR has the potential to induce measurable DNA damage under certain exposure conditions."

Input the table and conclusions Into the final technical report.

International Agency for Research on Cancer

World Health Organization

Reference to WHO/IARC is missing in 2018 Report

2016 NTP Report

"These findings appear to support the International Agency for Research on Cancer (IARC) conclusions regarding the possible carcinogenic potential of RFR."

NTP Technical Report 2018

?

Schwannoma in the Rats and Lymphoma in the Mice: By Chance?

Schwannoma in NTP-rats

Schwannoma in Ramazzini (Falconi 2018) -rats

Vestibular schwannoma (acoustic neuroma) in human case control studies (Hardell 2013)- people

Schwannoma (and mammary adenocarcinomas) in Ramazzini Study ELF+ Gamma (Sofritti 2015)- rats

Lymphoma = ENU in utero (Lerchl 2015, a replication Tillman 2010) – mice

Lymphoma, NTP - mice

Lerchl, Tillman and a summary of ELF need to be added to the NTP technical report.