

Can Extrapolation of “Omic” Data be
Applied **Appropriately** to Radio
Frequency Field Bioeffects
(and to RF Risk Estimation)?

Martin L. Meltz, Ph.D.
Kerrville, Texas 78028 USA

A Brief Introduction...

- Now retired (Professor with Tenure), after serving 35 years as a radiation biologist, 28 years in the Departments of Radiology and Radiation Oncology at the Univ. of Texas Health Science Center at San Antonio
- Approximately 18 years of experience in RF bioeffect research in the 350 – 3 GHz frequency range (funded by the U.S. Air Force and the AFOSR)
- Five years of research with 10 ns PEF exposures (funded by the AFOSR), including genomic and proteomic studies.

Hands on laboratory research with:

- Known and possible anti-cancer agents
- Known chemical mutagens and carcinogens
- Diesel engine exhaust fractions
- Flavonoids, chemicals found naturally in food
- Ionizing radiation (low LET)
- Ultraviolet light (germicidal)
- Hyperthermia
- Simultaneous chemical and RF exposures
- Simultaneous hyperthermia and RF exposures
- Sequential UV and RF exposure

Outline of Presentation

- Two general types of “Omic” assays
- Can “Omics” be applied to RF and EMF research?
- Hazard identification
- Has an “omic” system that could be applied to risk assessment been validated against known toxic agents, known mutagens and/or known human carcinogens?
- Has RF, in the frequency range of approximately 300 MHz to 3 GHz, been demonstrated to be toxic, genotoxic, or carcinogenic?
- Can “Omics” be used for risk estimation in the 300 MHz to 3 GHz RF range
- What role can “omics” play in RF research, when toxic activity has not been demonstrated?

Two General Types of “Omic” Assays

“Comparative Omic” Assays

“Perturbation Omic” Assays

“Comparative Omic” Assays

- “Comparative Omics” uses high through-put assays to establish patterns between two constitutive (pre-existing, chronic) physiological states
- This can be between cell types, tissue/organ types, normal/disease states, age, sex, etc)
- This should be controlled for any treatment or activity differences
- The range of potential use of comparative omics is likely limitless

“Perturbation Omic” Assays

- “Perturbation omics” uses high through-put assays to establish patterns when a constitutive state is altered due to an activity or treatment
- This can/should be examined as a function of level of activity or exposure dose (and preferably for several doses)
- This must be examined as a function of time after the perturbation activity or dose, because metabolic activity can be time dependent, and can also be transient.

“Perturbation Omic” Assays

Must be controlled for

- cell type and age
- tissue and organ of origin
- proliferation status (Go vs. cycling)
- cell cycle time and distribution. progression delay
- species, strain, sex, and age
- activity, hormonal status, diet and nutritional status
- drug or chemical exposure
- partial vs. whole body RF exposure (depth of penetration, orientation vs. source, etc.)
- Other?

Can “Omics” be Applied to RF and EMF
Research?

Pulsed UWB Sources and Cell Exposure

Cell Type Exposed: Continuously proliferating 244B human lymphoblastoid Cells

Source:	<u>10 ns PEF</u>
Cells Exposed in:	ODU Pulser
	Cuvette (0.1 cm)
	0.14 ml
244B Cell Density:	57,000,000/ml
Avg. Peak Power:	200 kV/cm (20 MV/m)
Frequency Range:	DC – 60 MHz
Pulse Rep. Freq.:	1/1.5 sec
Duration of Exposure:	< 1 min
Total Number of Pulses	25 (compared to 3 and 10)
Time of Assay Post-Exp.	2 and 24 hr post-exposure

Genomic Responses – 10 ns PEF 200 kV/cm (Pulser), 25 pulses

Objective and Experiment Protocol

- Cells were exposed in duplicate and incubated separately post exposure. The duplicate exposure flasks were combined and collected at **2 hr post exposure and 24 hr post exposure**.
- The total RNA was isolated from each sample of 2 combined flasks and prepared for hybridization onto **Affymetrix Human HGU133A (22,000 gene)** chips at the UTHSCSA Microarray Core Facility.
- 3 independent exposures were done on 3 different days, resulting in 6 exposed samples and 6 sham exposed samples (12 total).
- The RNA integrity of all 12 samples was checked at one time using the BioAnalyzer system at the Microarray Core Facility. As all 12 samples met criteria, the RNA samples were hybridized onto the chips, 4 samples at a time.
- After all 12 samples had been hybridized and scanned, the .CEL files were normalized using the RMA Pre-processor . They were then further analyzed using **GeneSpring Software v8.0**, and compared to the basic MAS analysis that was done by the Core Facility.
- Common genes from the lists will be chosen for further verification by RT PCR or another suitable method.

Summary for 25 Pulse Exposure Experiment

- At 2 hr post-exposure, 113 genes were changed compared to sham controls.
- 92 genes were increased, and 21 had decreased.
- 65 genes observed to change at 2 hr post after the 25 pulse exposure which are also changing in the 10 pulse experiment at 2 hr post exposure. Of these genes, all but one are increasing.
- At 24 hr post-exposure, 86 genes were changed compared to sham controls.
- 37 genes were increased, and 49 had decreased
- 2 genes observed to be increased at 2 hr after the 25 pulse exposure were still increased at 24 hr post-exposure. These were the genes *APAF1* and *PLSCR1*.

Genes of Possible Relevance

- For the experimental series of 3 pulses, 10 pulses and 25 pulses post-exposure to 200 kV/cm, only 3 genes were increased by a ratio of 2 fold or greater in all 3 groups at 2 hr post exposure. Those genes are *NR4A2* (Nuclear Receptor subfamily 4, group A, member 2); *RGC32* (Response gene to complement 32); and *CCL4* (chemokine (C-C motif) ligand 4). These 3 genes are in the gene type “protein coding”.
- *MBIP* was the only gene observed to change at 2 hrs after both 10 and 25 pulse exposures. In both cases, a decrease was observed.
- 65 genes were found to have changes after both 10 and 25 pulse exposures, at 2hr post exposure. Of those 65 genes in common, possible relevant genes, each of which were increased compared to sham controls, were *FOS*, *JUN*, *EGR1*, *EGR2*, *EGR3*, *EGR4*, *MCL1*, *FOSB*, *DUSP1*, *DUSP4*, and *GADD34*. Some of these genes have been studied in our laboratory, for confirmation, using other assays.

Conclusion: Yes, “Omics” Can be Applied to EMF Research

- There is a reportable genomic response (and also a proteomic response, not shown) to the specific EMF exposure, which is dose and time dependent (and transient).
- The relationship of these responses to the level of cell death can be reported, with subsequent hypothesis driven research being an option for relating the changes to the level of cell killing (or survival).
- However, the changes may also partially be due to changes in cell proliferation, cell cycle alteration, other metabolic changes, etc. None of these parameters were measured (or therefore controlled for) in these experiments.
- There are major issues with respect to correlation in time of proteomic changes and genomic alterations

But a major issue for the appropriate use of
Omics to study RF Risk is Hazard
Identification

Hazard Identification

- Epidemiological Studies
- *In Vivo* Animal Bioassays
- Short-term *In Vitro* Cell and Tissue Culture Tests
- Structure-activity Relationship Analysis
- Mechanism Information

Some Established Tests *

“Appendix A contains a brief summary of the test systems and assays most commonly used to evaluate whether a chemical, radioactive, or biological agent poses a hazard to human health or the environment.

- Long-term rodent bioassays
- Salmonella Mutagenicity Assay (Ames Test)
- In Vitro cytogenetic assay
- Unscheduled DNA synthesis
- Mammalian Cell Transformation
- Exotoxicity Laboratory Tests
 - Short-term- morbidity. behavioral or physiological
 - Long-term: growth, reproduction, maturation, spawning, hatching, survival, behavior, and bioaccumulation

* “Risk Analysis: A Guide to Principles and Methods for Analyzing Health and Environmental Risk” JJ Cochrane and V.T. Covello U.S. Government 1989

Appendix A: Hazard-Identification Test Summary Sheet*

- Name of the Test
- Endpoints (specific physiological or metabolic effects) measured by the test (For RF, ???)
- Health and environmental hazards inferred from the results of the test (For RF, ???)
- Summary of the protocol for the test
- Major sources of uncertainty in the test (For RF, ???)
- Accuracy of the test in terms of human effects and correlation with other laboratory tests (For RF, ???)
- Degree to which the test has been developed (For RF, ???)
- Resources required to conduct the test
- Literature references that provide more detailed information about the test

* "Risk Analysis: A Guide to Principles and Methods for Analyzing Health and Environmental Risk" JJ Cochrane and V.T. Covello U.S. Government 1989

Has an “omic” system that could be applied to risk assessment been validated against known toxic agents, known mutagens and/or known human carcinogens?

- Validation was a major activity undertaken as part of the U.S. EPA's Gene-Tox program, when different investigators attempted to demonstrate that their assay system was the most suitable for indicating the possible mutagenicity, and therefore the possible carcinogenicity, of chemicals and drugs either in or being developed for introduction into the ecosystem
- To my knowledge, the answer with respect to the question above is that there has been no such validation.

Has RF in the frequency range of approximately 300 MHz to 3 GHz been demonstrated to be toxic, genotoxic or carcinogenic?

For RF field exposures, at SARs which do not raise the temperature of a biological system above its physiological level,

While there are published reports to the contrary, the scientific weight of evidence supports the following statements:

That there is/are:

- No increases or decreases in the rate of cell proliferation
- No stimulation/initiation of cell proliferation
- No alterations in cell cycle distribution
- No decreases in cell viability (or apoptosis)
- No induction of DNA strand breaks
- No induction of DNA repair synthesis
- No alteration in UV induced DNA repair synthesis

- No induction of chromosome aberrations
- No increase in chemical induced CAs resulting from simultaneous RF exposure (not attributable to a temperature increase)
- No induction of micronuclei
- No induction of sister chromatid exchanges (SCEs)
- No increase in chemical induced SCEs resulting from a simultaneous RF exposure (not attributable to a temperature increase)
- No induction of mutations at the TK+/- locus in L5178Y cells

- No increase in chemical induced TK+/-mutation resulting from a simultaneous RF exposure (not attributable to a temperature increase)
- No induction of mutations in the Ames bacterial mutation assay
- No induction of tumors in chronic rodent bioassays
- No induction of lymphoblastoid lymphomas in transgenic (sensitized) rodents

- No sustained physiological alterations in organ function or biochemical endpoints in a large number of organs during or resulting from a chronic rodent exposure
- No decrease in lifetimes in chronic rodent bioassays
- No weight loss in chronic rodent bioassays

Can Extrapolation of “Omic” Data be Applied Appropriately to Toxic Radio Frequency Field Bioeffects, in particular to Risk Estimation?

- The cost for performing high through-put “omic” studies is bordering on prohibitive
- There may or may not be a cellular physiological effect resulting from the omic alteration
- Any omic response may be transient, and while it may be necessary for a subsequent metabolic or physiological alteration to occur, it may not be sufficient for that alteration to occur.

- There are an extensive number of biological, dose and time variables to be dealt with in beginning to understand and interpret the meaning and significance of “omic” changes
- The validation of “omics” has yet to be accomplished
- The cellular physiological alteration could result in beneficial effects to human physiology, or be of no significance, rather than leading to an adverse consequence.

There is an overwhelming weight of laboratory evidence, at the current allowable levels of RF exposure and sometimes at higher levels of exposure, of the absence of biological effects indicative of cellular alterations, toxicity, genotoxicity and carcinogenicity

Conclusion

The application of “Omic” technologies to risk assessment for RF field exposures, without establishing a true associated hazard, is premature, and until a definite hazard is established, such an application is inappropriate for public health risk estimation purposes.

What role can “omics” play in RF and EMF research, when toxic activity has not been substantiated or is not present?

If an “omic” or biological effect of a given RF or EMF exposure is observed, there might be some mechanism, previously unknown to physics and biology, by which that specific RF exposure signal is recognized by a cell or tissue

- The dose and time dependence of any “omic” alteration could be compared to the dose and time dependence of any identified biological effect

- A threshold for the omic and/or biological response could possibly be determined
- Further investigation could lead to an understanding as to how and where in the cell or tissue the signal is detected, and
- how the chain of molecular and/or physiological changes is initiated.

I wish to thank the hosts and sponsors of this workshop for inviting me to attend, and to present my views on an important and challenging topic.