ADVANTAGE, CHALLENGES AND LIMITS OF EXPERIMENTAL STUDIES

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EXPERIMENTAL STUDIES ARE USED TO ASSESS RISK TO CHEMICAL, PHYSICAL OR BIOLOGICAL AGENTS

…considering the EM fields

- Advantages
- Challenges
- Limits

Some founded EMF projects based on experimental studies:

EMF RAPID – Electric and Magnetic Fields Research and Public Information Dissemination Program; GUARD; EMFnEAR; RAMP 2001; CEMFEC, Reflex

EM STUDIES AN OPEN PROBLEM

- Large number of studies but controversial results
- Limited number of studies in relation to particular endpoints

Are they really able to give conclusive information???

ADVANTAGES – DRAWBACKS

- Experimental studies are the most accurate way to learn the effects of EM fields in living matter
- Allow dose - response studies

In Vitro Experiments

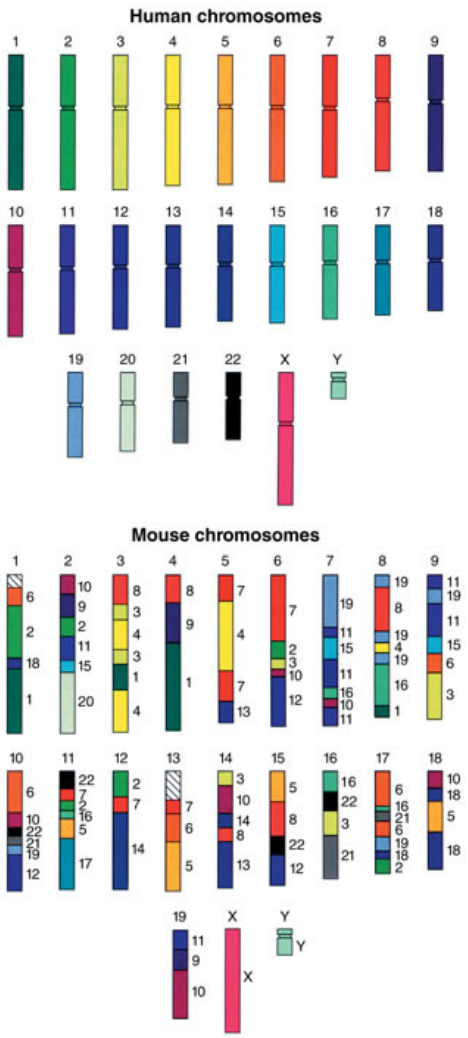
•are fast and cheap
•permit mechanistic studies
•use of human cells or "target" cells
•allow more controlled exposure
•allow real time data acquisition
•do not present the organism complexity
•do not resemble to the organ of origin

In Vivo Experiments:

•can reveal side effects like organ failure, as in tumours
•is accurate enough to test whether EM fields are even safe enough for humans.
•inter-individual variability
•are expensive
•ethical issues
•animal are kept in unnatural conditions

Human Experiments: *the best model for EM fields studies!*
inter-individual variability, acquisition procedures, ethical issues

IN VIVO: MOUSE MODELS



Genome homology between human and mouse

Similarities between mouse and human genes 70-90%

- Reproduce rapidly
- Short life spans
- Easy to handle
- Can be genetically manipulated

IN VIVO: MOUSE MODELS

Suitable animal model

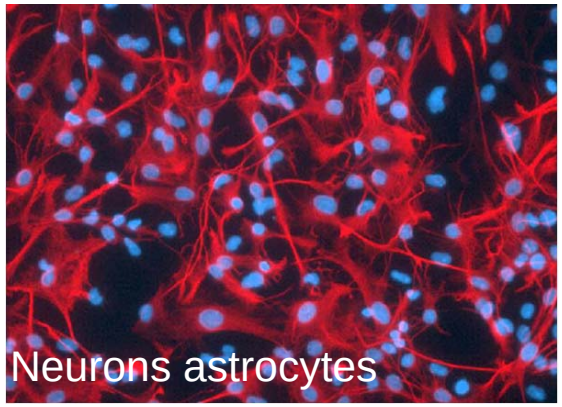
- Is the model chosen appropriate to address the question raised?

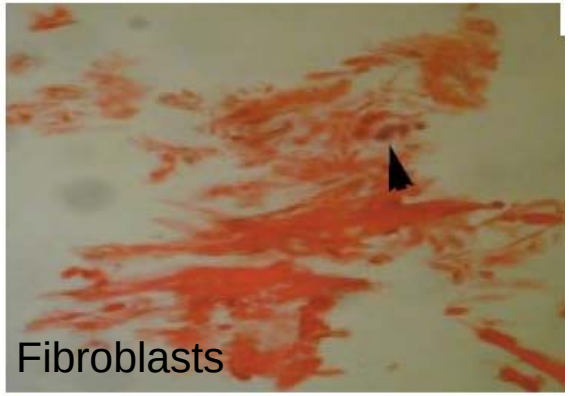
Conventional mice:
Inbred mice
Out-bred mice

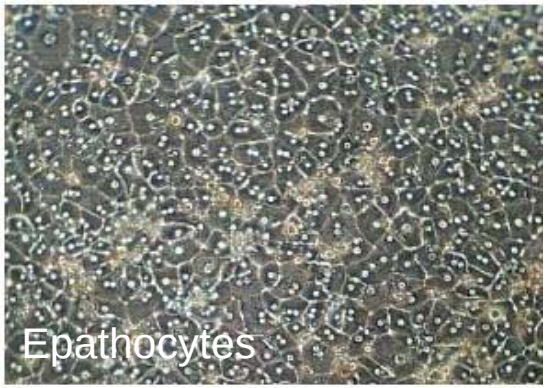
Genetically selected/modified animals

IN VITRO: CELL MODELS

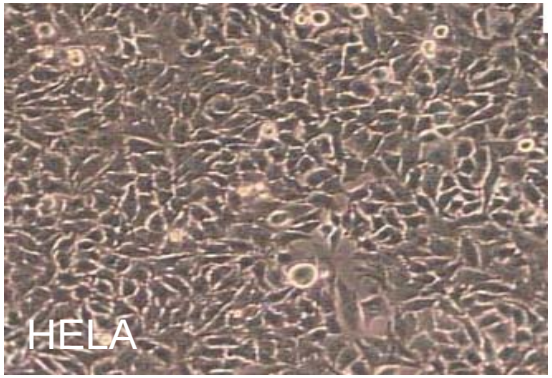
Primary cultures: derived directly from living donors in a few culture passages they lose vitality

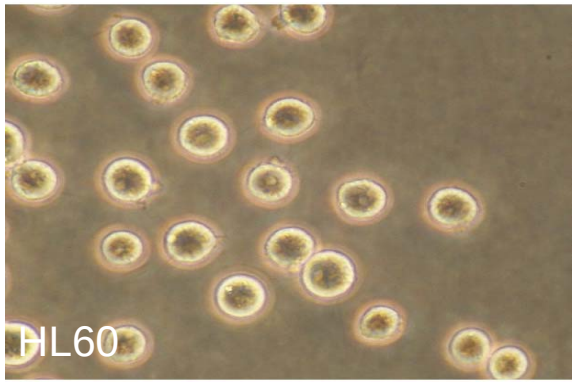


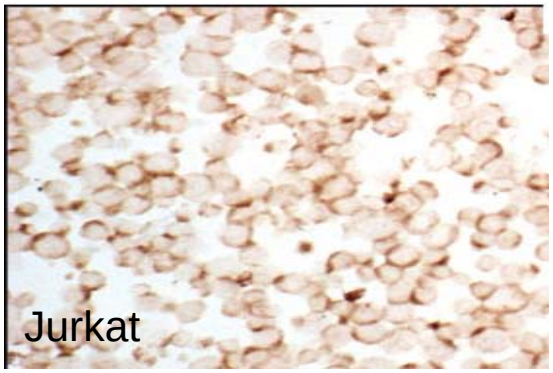




Immortalized cultures: maintain their vitality indefinitely but show peculiar characteristics of tumour cells



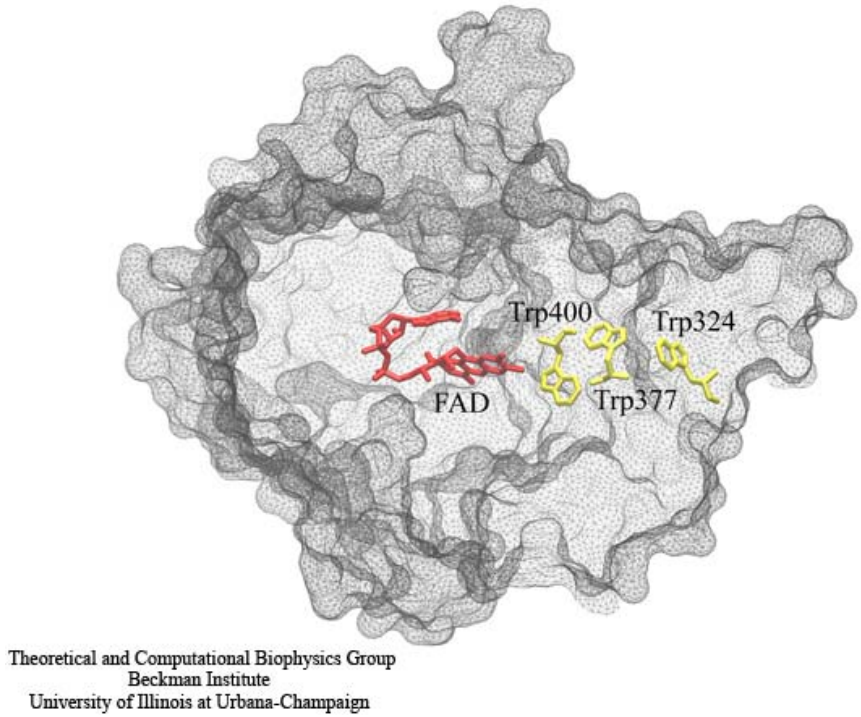




IN VITRO: TESTING MECHANISTIC HYPOTHESES AT SUB-CELLULAR AND MOLECULAR LEVEL

Radical pair mechanism: the most plausible to explain the effects of weak MFs. Chemical reaction affected the level of free radicals, this reaction is increased in low MFs and decreased in high MFs.

Biological relevance of such supposition to be established



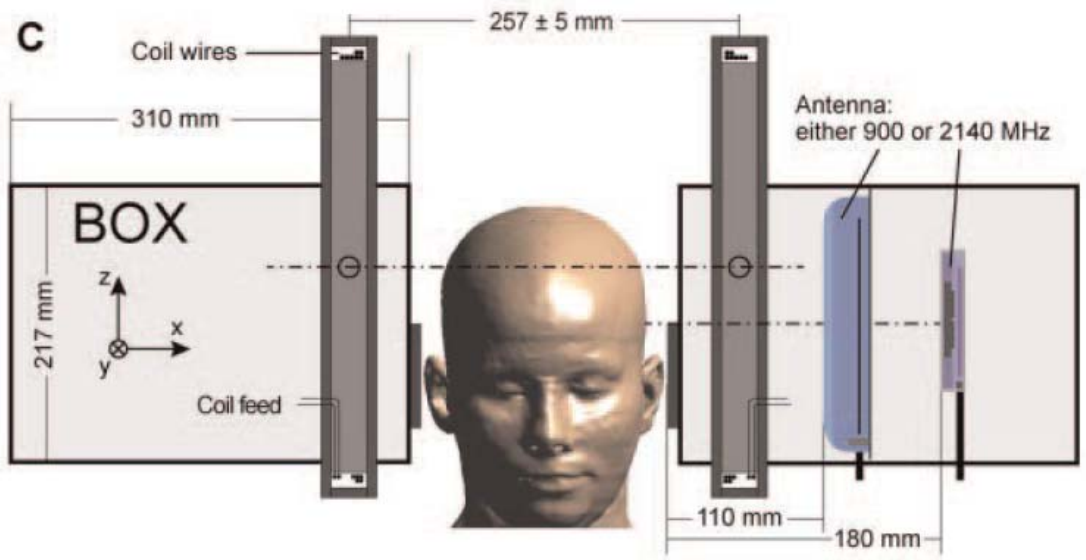
Blue-light photoreceptor cryptocrome has been proposed as a suitable molecule to verify such hypotheses

T. Ritz et al., 2010

Maeda and Henbest et al., 2008

HUMAN EXPERIMENTS: NO APPROXIMATIONS OF THE BIOLOGICAL MODEL

…however limited biological endpoints can be evaluated as for example: cognitive and provocational tasks or EEG recordings



Murbach et al., 2012

Volunteers have to be found, it is needed the approval of ethical committees

HUMAN AND IN VIVO STUDIES

Inter-individual variability

- Variability in exposure dose among individuals of the same group
- Variability in values of biological parameters and in response to stressor

CHALLENGES

- Difficult interpretation of results
- Identification of a responsive model (in vitro in vivo)
- Choice of the best testing procedures
- Replication (different and independent laboratories)
- Positive and negative control, blind
- Dose and exposure assessment
- Use of appropriate statistical tools
- Multi-sources and all life long exposures
- Co-exposures
- Whole uncertainty evaluation

In Vitro Experiments:

•Translate results from cells to organisms

In Vivo Experiments:

•Age dependency

•Multigenerational studies

Human Experiments:

•Standardization of exposure

•Double blind

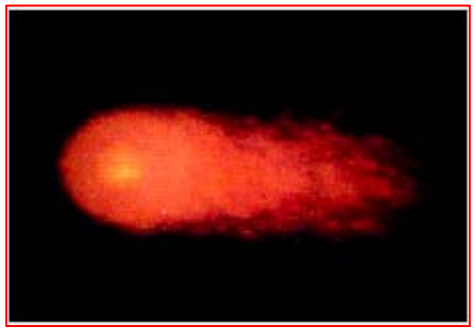
•EMI/EMC problems of EMF with other instrumentation.

•Study group

CHOICE OF THE BEST TESTING PROCEDURE

DNA strand breaks evaluation:

Comet Assay: damages of transient nature, it is simple chip fast very sensitive



a test for genotoxicity the fixed DNA damage must be assed by classical:

Micronuclei Test

Chromosomal Aberration

Different tests for a same endpoint cannot produce the same results when performed in different laboratories

REPLICATION

Some examples of the past:

Original work	Experimental system	Replication	Results	
Smith et al, 1987	Diatom motility	Parkinson and Sulik, 1992 Prasad et al, 1994 Saalman et al, 1991 Davies et al, 1993, 1994 Reese et al, 1991	Failure Failure Failure Partial Partial	EMF Rapid 1994-1998 Various end-points
Shuvalova et al, 1991 Markov et al, 1993	Calmodulin dependent myosine phosphorylation	Hendee et al, 1996 Coulton et al, 1997	Failure Failure	
Goodman and Shirley-Henderson, 1991	MYC and β-actina transcription inHL60 cells	Lacy-Hulbert et al, 1995 Saffer and Thurston, 1995	Failure Failure	
Lindstrom et al, 1993	Ca+2 oscillation in Jurkat T cells	Walleczeck et al, 1994	Failure	
Litovitz et al, 1991, 1994	ODC activity in mouse L292 cells	Azadniv at al, 1995	Failure	
Repacholi et al., 1997	E mu-Pim1 trans-genic mice	Utteridge et al., 2002 Oberto et al., 2007	Failure	cancer
Russian Ukrainian papers from 1974–1986	• Complement fixation test (CFT) • Antigen in rat sera using ELISA tests	Poulletier de Gannes et al., 2009 Grigoriev et al., 2010	Failure Partial	immune system
Salford et al., 1994, 2003 Finnie et al., 2001, 2002 Tore et al., 2001	• Albumin extravasation • Albumin leakage with labeled fluorescent proteins	McQuade et al., 2009 Kuribayashi et al., 2005	Failure	BBB

IN VIVO EARLY LIFE EXPOSURE

- Exposure to WiFi signals (2.45 GHz) during pregnancy

  - Studies on male and female offspring at early (5 wks) and late (26 wks) time points
  - Pregnancy outcome
  - B cell development and antibody production(early and late time points)
    - Sambucci et al, Radiat Res 174:732, 2010
  - T cell development
    - Laudisi et al, Bioelectromagnetics, in press, 2012

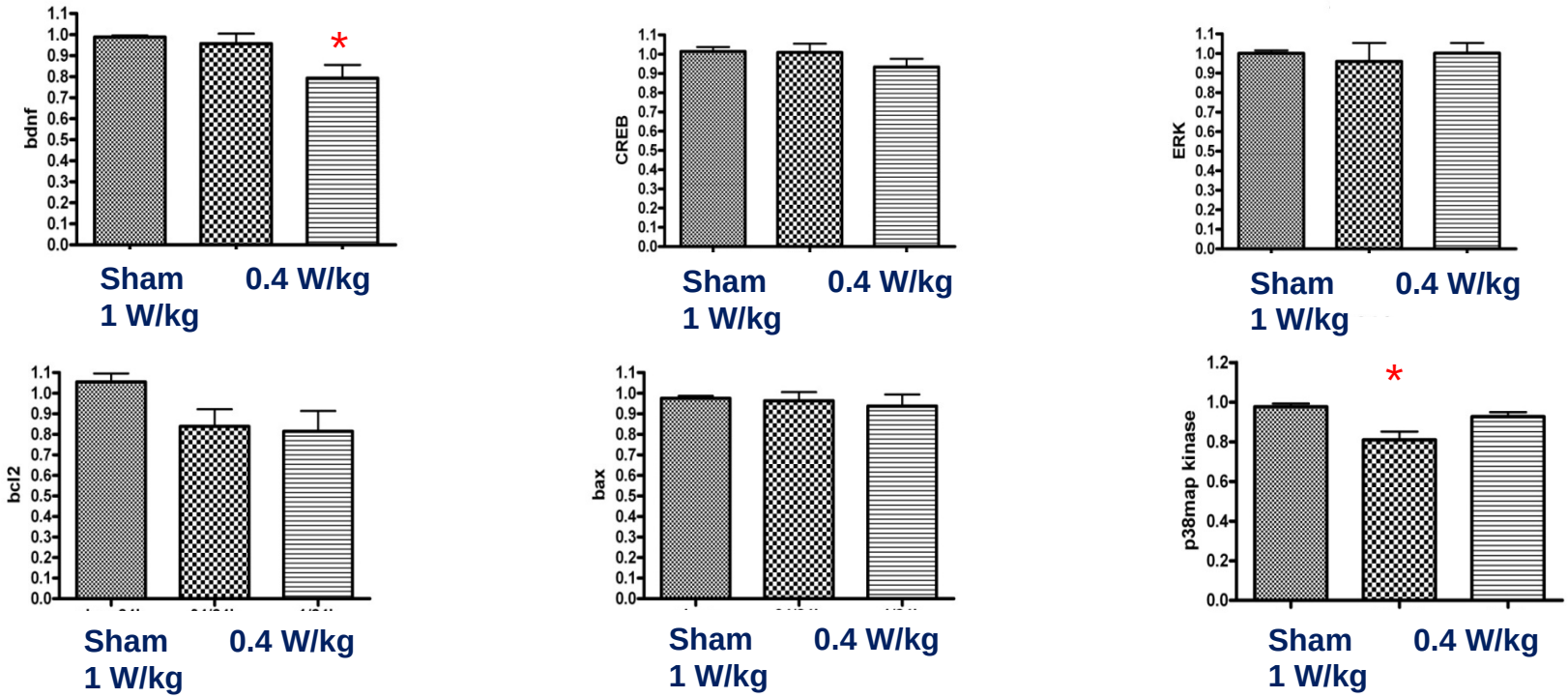
- Exposure of newborn animals to WiFi signals

  - Exposure first 5 wks of age
  - B and T cell maturation
    - Prog. Bioph Mol Biol 107: 393, 2011

IN VITRO: INTERPRETATION OF EXPERIMENTAL EVIDENCE

RESULTS OF 24H OF EXPOSURE

- Significant reduction in the levels of p38 Map kinase with the dose of 0.4 W/kg ($p<0.05$), but not at the dose of 1 W/kg.
- Conversely, the 24h exposure caused a reduction in BDNF levels only with 1 W/kg dose ($p<0.05$).
- No effect has been observed on the expression levels of CREB, ERK, BAX and Bcl-2.



Statistically significant = biologically relevant ?

What does it mean at organism level?

IN VITRO AND IN VIVO:CO-EXPOSURES

+

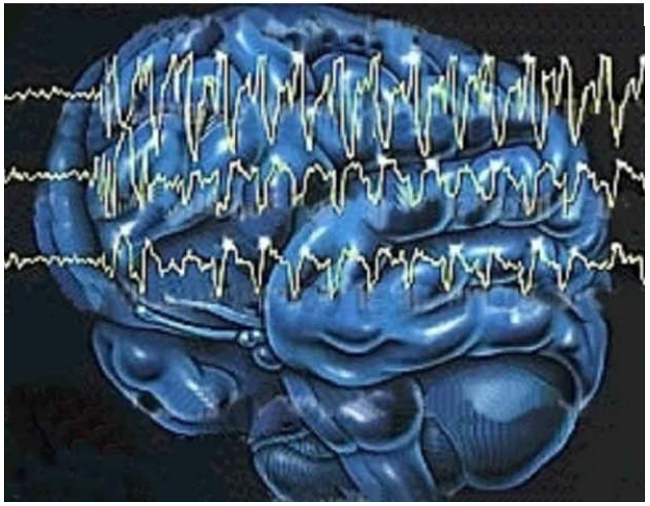
Juutilainen et al., 2006, Brescia et al., 2009, Zeni et al., 2009

- ***RF studies on co-exposures are mostly negative for genotoxic or carcinogenic effects (other end-points ?)***
- ***Majority of the ELF studies (65%) are positive, research challenge for mechanism identification***

HUMAN STUDIES: EMI/EMC

Bias and uncertainty due to acquisition procedures in EEG recordings

Regel and Acherman, 2011 Valentini et al., 2007; Kuster et al., 2004; D'Andrea et al., 2003

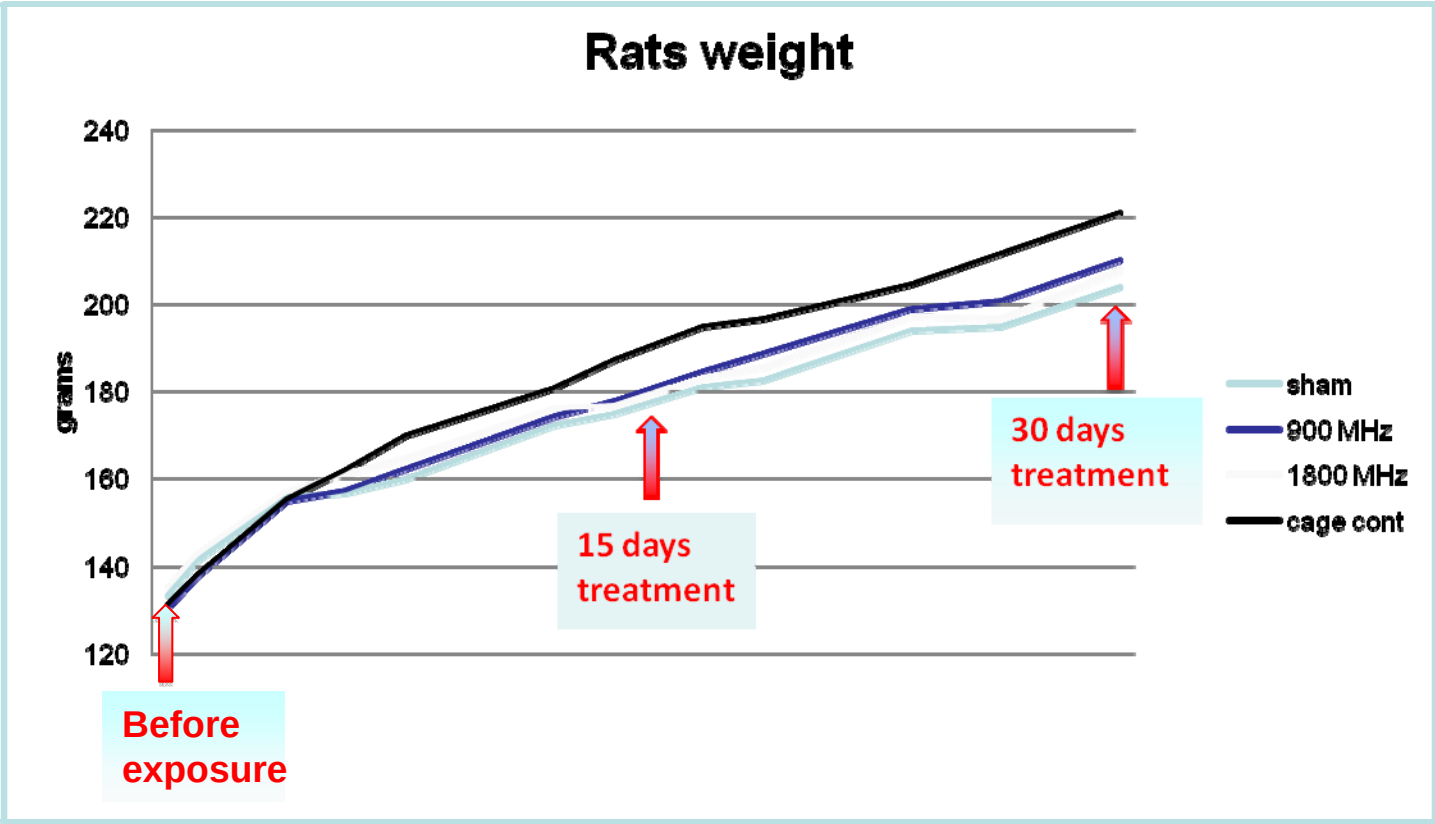


- Electrodes presence, it is needed to assure minimum coupling, possible generation of spurious signal and artefacts due to RF current induction
- Validation and replication of original findings in the same and different laboratories
- Varying the measurement procedures of the observed endpoints if possible

LIMITS

- Confounding factors, the expected effect seems to be small
  - Ambient control
  - Stress
  - Animal strains genetically prone to disease development
  - Side effects on positive control (drugs)
  - Water and food
- Cost and time
- Limited number of endpoints can be studied
- In all biological systems are present
  - Interactions
  - Effects on not targeted organs
  - Compensation/homeostasis, co-exposures
- Dosimetric limitations (e.g. in vivo experiments of freely moving animals)

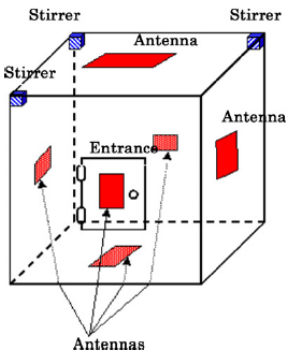
THE STRESS FACTOR



Effect of restraint on rat weight

DOSIMETRIC ASSESSMENT

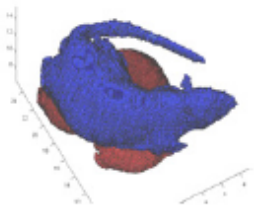
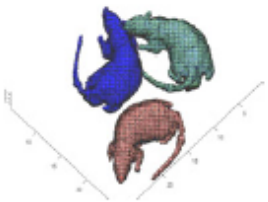
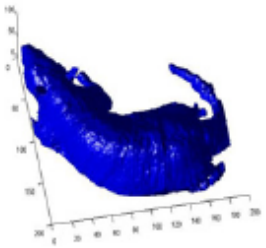
The reverberation chamber



Dosimetry cannot realistically test all the exposure configurations due to different mice positions, ages, growth rate…

Wu et al., Phys. Med. Biol., 2010

Table 3. Occurrence of various behaviours at different ages.

	Group with adult	Group without adult	Single
Configuration			
0–4 days after birth	95%	5%	
6–10 days after birth	65%	30%	5%
13—19 days after birth	33%	33%	33%
20—30 days after birth		50%	50%

THE RESEARCH QUALITY

The EM point of view

- To provide exposure set-up dosimetry both numerically and experimentally
- To provide uncertainty evaluation both for measurements and numerical calculations
- To provide monitoring of local and environmental temperature as well as for incident and reflected power

The biological point of view

- To use the appropriate animal model according to 609/86 EU instruction (Refinement, Reduction, Replacement) or suitable cellular targets
- To replicate the experiments in order to obtain statistically significant results
- To perform sham and blind experiments and, if possible, include positive and negative controls

DATA WEIGHT OF EVIDENCE

Data results will have different weight of evidence depending on the addressing of the mentioned issues,

however minimal requirements for a sufficient quality of any biological experiment should always include:

- Accurate exposure setup dosimetry both numerically and experimentally
- Uncertainty evaluation
- The use of a sufficient number of samples to get good statistics
- The repetition of biological assay for appropriate data statistics and interpretation
- Sham and blind experiments and if it is possible positive control use

CONCLUSIONS

EXPERIMENTAL STUDIES with CELLS → **Mechanism**

✓Good statistical number
✓Repeatibility,
✓Provide co-exposure,
✓No complexity
✓many good quality, but still some with inadequate exposures or dosimetry

EXPERIMENTAL STUDIES with ANIMALS → **Confirm of bioeffects; physiopathology issue**

✓variety of models used, exposures, signals
✓mainly rodents,
✓many good quality, but still some with inadequate exposures or dosimetry

EXPERIMENTAL STUDIES with HUMAN

✓Specific end-points,
✓Set of the experimental protocol,
✓Statistical power,
✓Inter individual variability