

Madrid, 26 Oct 2012

Conocimientos Actuales en Radiobiología (Jornada Técnica)



Efectos biológicos de las RNI: semejanzas y diferencias con las RI
(Biological effects of NIR: similarities and differences with IR)

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SaludMadrid



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"The new scientific paradigm is that to understand life we need a dual approach: molecular and electro-magnetic. The universe is made of matter and waves. Thus, molecules and cells interact through contact and waves. Probably physics will soon change our current concepts in molecular and cell biology."

Luc Montagnier: in DM; June 2010



LUC MONTAGNIER

Nobel Prize (2008) for the identification of HIV (AIDS)

The bioeffects of NIR

An approach from the biomed sciences

Epi / Observational



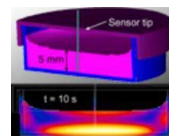
Experimental in humans



Experimental in vivo



Experimental in vitro



Mechanisms



The experimental approach to the effects of NIR on biological models has a double "translational" aspect

EXPERIMENTAL 1A (RP against RF)

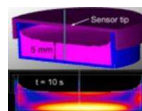
- Trillo et al., Cytostatic Response of NB69 Cells to Weak Pulse-Modulated 2.2 GHz Radar-Like Signals. BEM Journal (2011)

EXPERIMENTAL 1B (RP against LF)

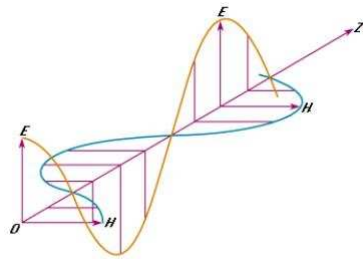
- Úbeda y col., Weak environmental fields and semicircular lipotrophy. Seguridad y Medio Ambiente (2011)
 - Martínez et al., The Proliferative Response of Human Neuroblastoma Cells to a 50 Hz MF is mediated by ERK1/2 Signaling. Cell Physiol Biochem (2012)

EXPERIMENTAL 2 (E-M therapies)

- Hernández-Bule et al., RF currents exert cytotoxic effects in NB69 human neuroblastoma cells but not in peripheral blood mononuclear cells. Intl. J. Oncol (2012)



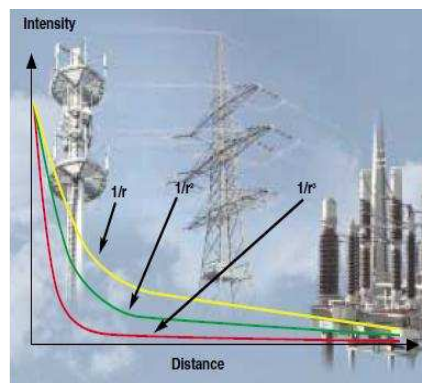
NIR: Electromagnetic fields



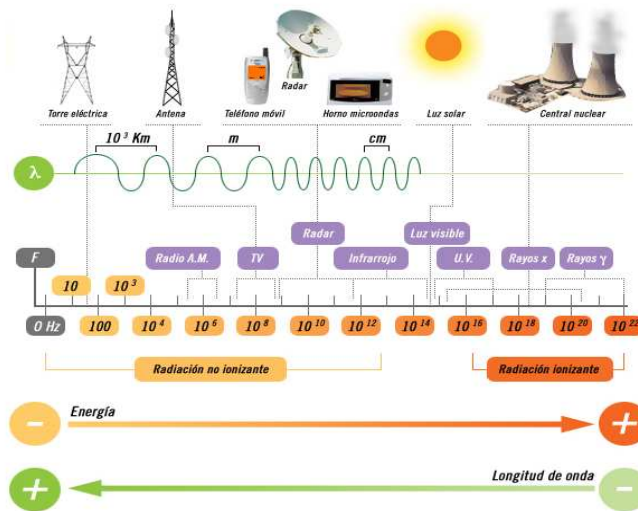
- EM signals with components E and H perpendicular
- Light speed
- The higher the frequency...
 - Higher energy
 - Lower penetrability

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Intensity decays with distance



The electromagnetic spectrum



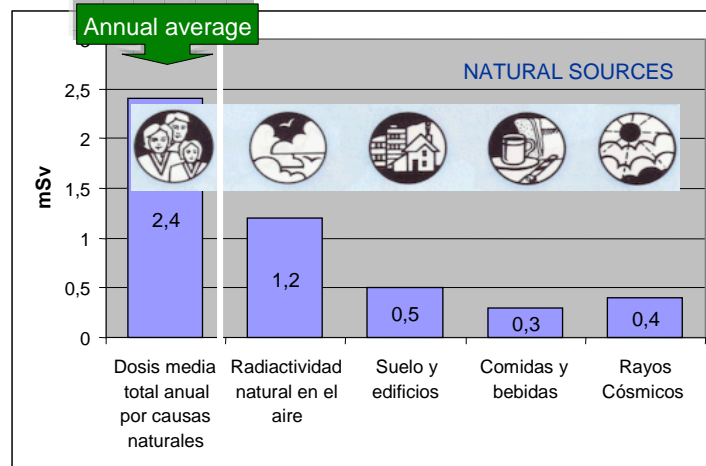
IONIZING RADIATION

vs NON-IONIZING



- **Ionizing radiation (RI)** has enough energy to remove an electron from an atom, producing an ion. Direct genotoxic action (40%) or indirect (free radical: 60%)
- **Non-Ionizing radiation (NIR)** lacks the energy to ionize: No direct genotoxicity. But "indirect" effects reported: on FR, DNA repair, gene expression plus other, non genotoxic effects. **Not considered for exposure limits in PR**

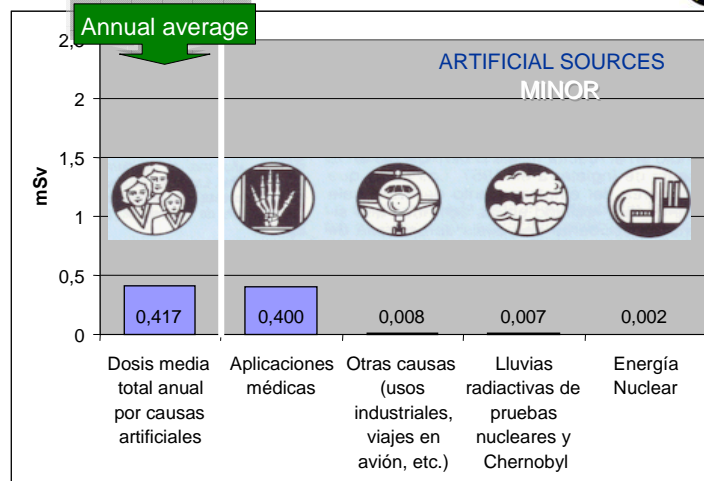
ANNUAL DOSES OF NATURAL IR



Contribution of different natural sources of radiation to the total annual average dose received by the world population (UNSCEAR, 2000)

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ANNUAL DOSES OF ARTIFICIAL IR



Contribution of different artificial sources of radiation to the total annual average dose received by the world population (UNSCEAR, 2000).

Anyway: Precautionary Principle applies to any dose (**ALARA**)

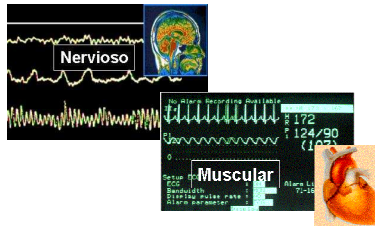
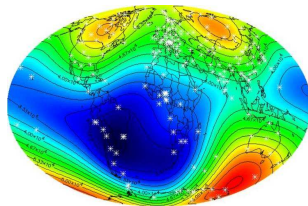
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NATURAL SOURCES OF NIR

Few, weak. Biosystems adapted



- Solar
 - RF + Visible light
 - IR
 - UV
- Geomagnetic
- Electric storms
- Bioelectricity
 - Nervous (EEG, MEG)
 - Muscular (EMG)



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ARTIFICIAL SOURCES OF NIR MAJORITY

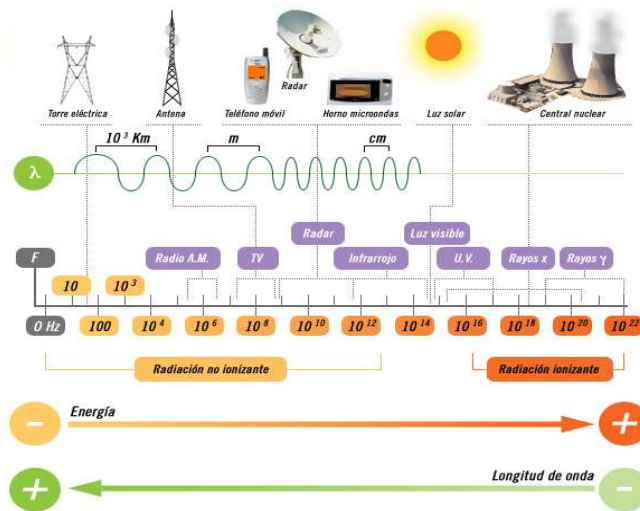
Many, powerful, recent (Biosystems unadapted?)



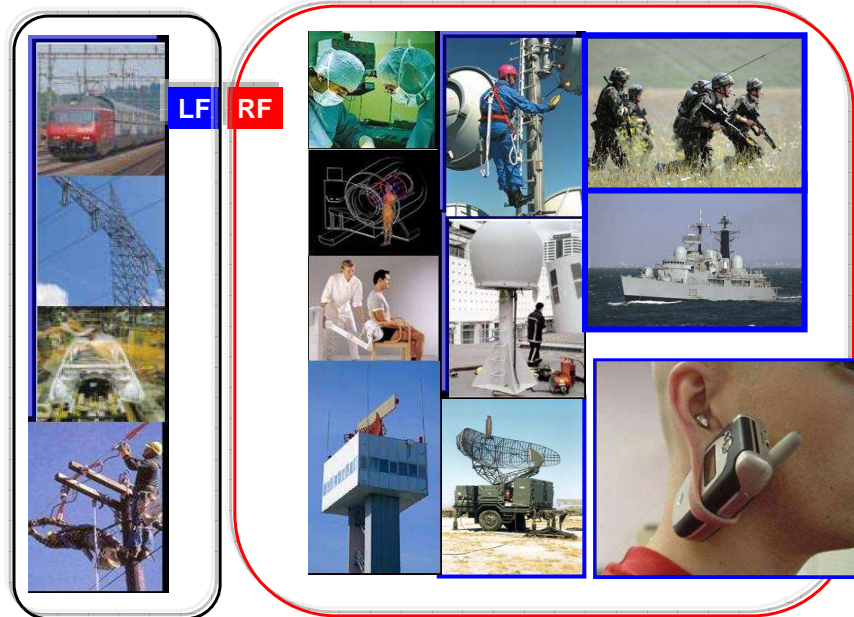
- Static: Magnets, coils, Maglev...
- Power frequency
 - Power lines
 - Transformers
 - Wiring
 - Electric equipment
- RF radiocommunication
 - Radio AM, FM, TV, GSM, UMTS, Wi-Fi, Wi-Max, radar...
- MW: Home/industry
- Medicine
 - MRI
 - Transcranial M.S
 - Magnetotherapies
 - Electrothermal T
 - Diathermia/MW
 - Laser
 - IR
 - UV

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NIR in energy, telecommunications and medicine: A recognized benefit and projecting into the future



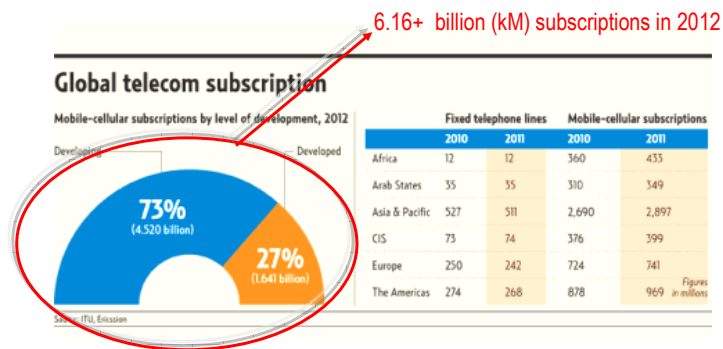
Uncontrolled exposure to NIR has become ubiquitous and almost universal: An emerging risk?





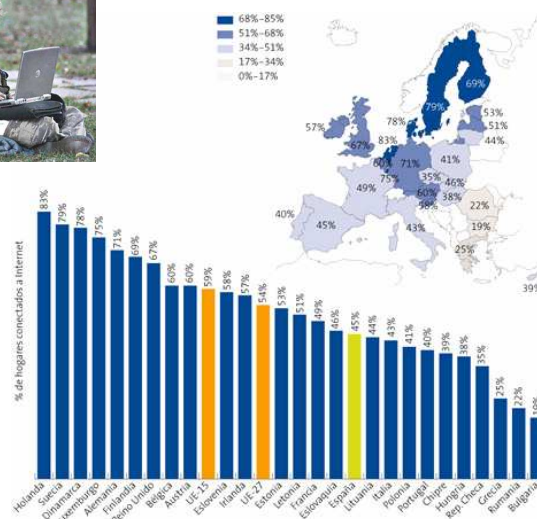
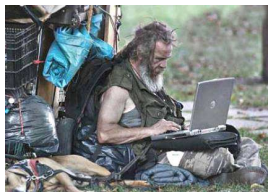
Should specific NIR have any adverse effects...

- The individual risk might be low (9/100.000 year)
- But social/health impact at the national, international and global scales, potentially serious



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Other wireless technologies: NOT studied



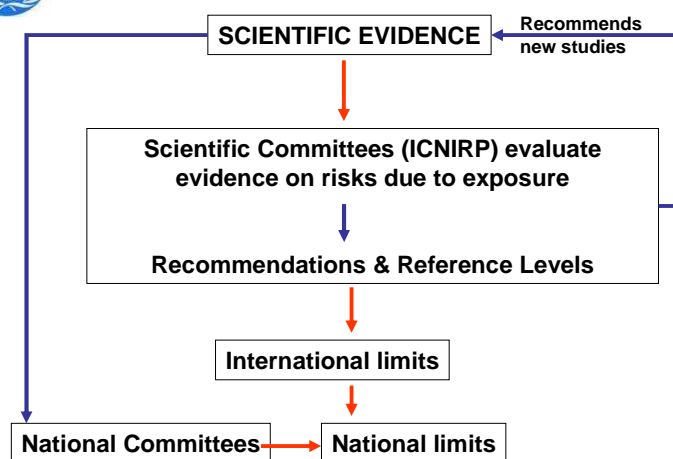
Criteria for radiological protection against NIR



The ICNIRP Criteria

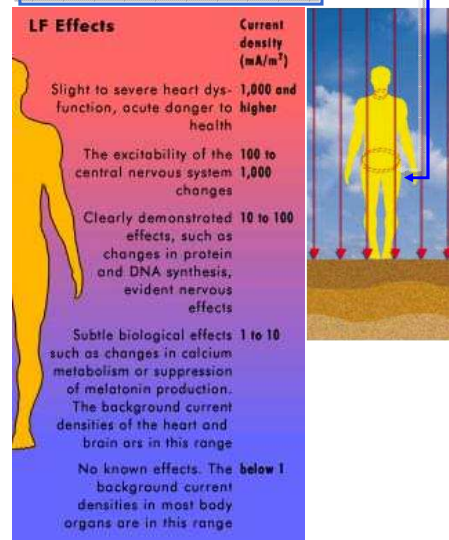


Establishing standards for NIR exposure (ICNIRP 1998)



Protection against immediate, harmful effects of LF Radiation

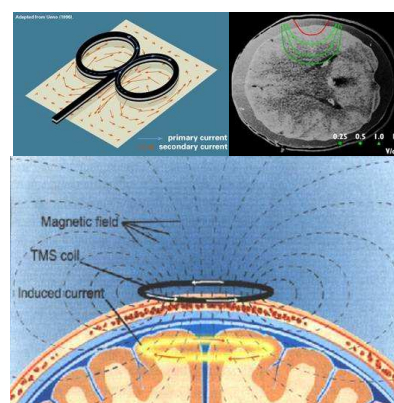
LF: Eddy Currents



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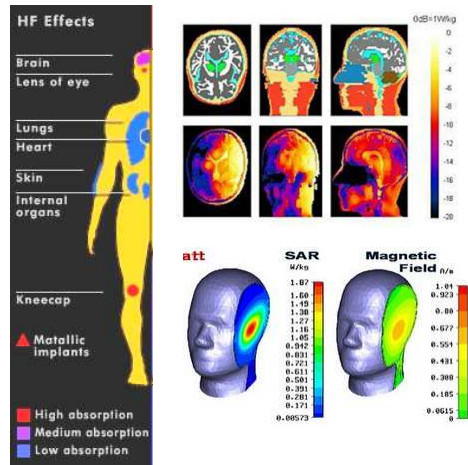
LF: Transcranial Magnetic Stimulation (MTS)

- EM spectrum: 1-25 Hz
- MF: 2 T (0.5 T at cortex)
- Depolarizes neuronal populations. Short-term:
 - On motor cortex: Motor response
 - On occipital cortex: Phosphenes
 - Other areas: Behavioral / cognitive
- MTS repetitive: Changes in synaptic efficacy? Long-term
 - Enhanced excitability
 - Depressed excitability



http://www.youtube.com/watch?v=FMR_T0mM7Pc&feature=player_embedded

Protection against immediate harmful effects of RF: $\Delta T > 1^\circ\text{C}$

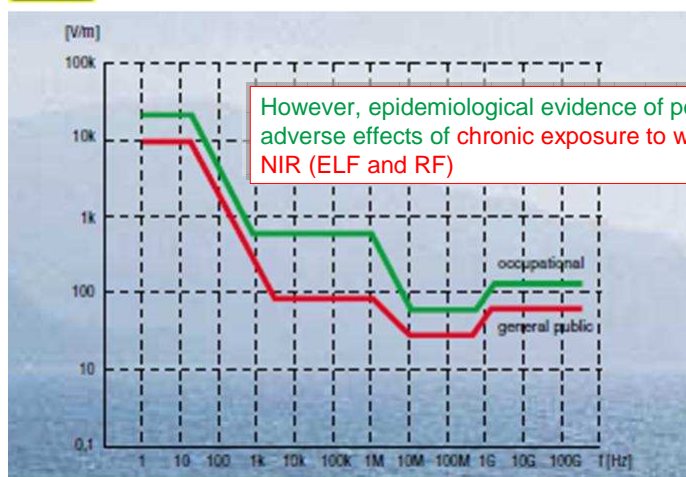


<http://www.youtube.com/watch?v=V94shlqPISI&feature=BFa&list=ULI2FerVKFv5E&index=2>

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NIR: ICNIRP Reference Levels (1998) for short-term exposure, public/occupational



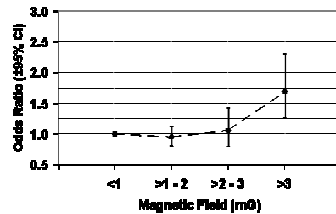
However, epidemiological evidence of potential adverse effects of chronic exposure to weak NIR (ELF and RF)

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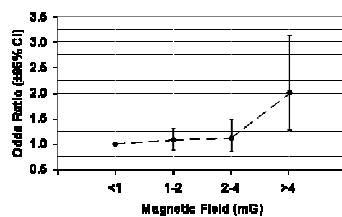


Epidemiology of childhood leukemia and residential exposure to 50-Hz, $B \geq 0.3 \mu\text{T}$ MF, (ICNIRP RL = $100 \mu\text{T}$)

Pooled analysis: Greenland et al., 2000



Pooled analysis: Ahlbom et al., 2000



More recent epidemiology reinforces those results: IARC 2002...



International Agency for Research on Cancer
Centre International de Recherche sur le Cancer

classifies ELF EMF as "possible carcinogens", Class 2B

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Epidemiology of head/brain tumors in M.Ph users : Timeline

- 2007 – 2010 ► Various epidemiological studies (and pooled analysis) show signs of increased tumor risk (glioma & meningioma) in long-term users ($T > 10$ years).
- May 2010 ► First results of INTERPHONE published in *International Journal of Epidemiology*
- 2011:
 1. May: Several international organizations admit the possibility of long-term effects of chronic exposure to RF subthreshold. Propose to modify the limits for RP and/or apply the Precautionary Principle
 2. Other results of INTERPHONE and new epi. studies are published that reveal additional indications of carcinogenic potential of RF signals emitted by phones.
 3. June: After reviewing recent epi. and experimental evidence, IARC (WHO) includes RF in its classification of carcinogens, within the category of "possible carcinogens (2B)" based on an increased risk of glioma.
 4. October: An epi. study (cohort) finds no relationship between prolonged use of M.Ph and incidence of brain tumors.
- 2012:
 - May & Aug (some authors in common): Pooled analysis: Significantly increased risk of glioma, particularly in subjects with first use before the age of 20

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World Health Organization

Protection against exposure to weak NIR : two approaches

International Agency for Research on Cancer



World Health Organization

– **IARC-WHO: ELF (PF; 2002) y RF (radiocommunication; 2011) Possible carcinogens, class 2B**



- **ICNIRP: Mechanisms unknown** => Evidence **not considered** to set limits for protection of workers or public



CONTROVERSY

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RP: A recent discipline



International
Radiation
Protection
Association
Founded 1965



Founded
1984



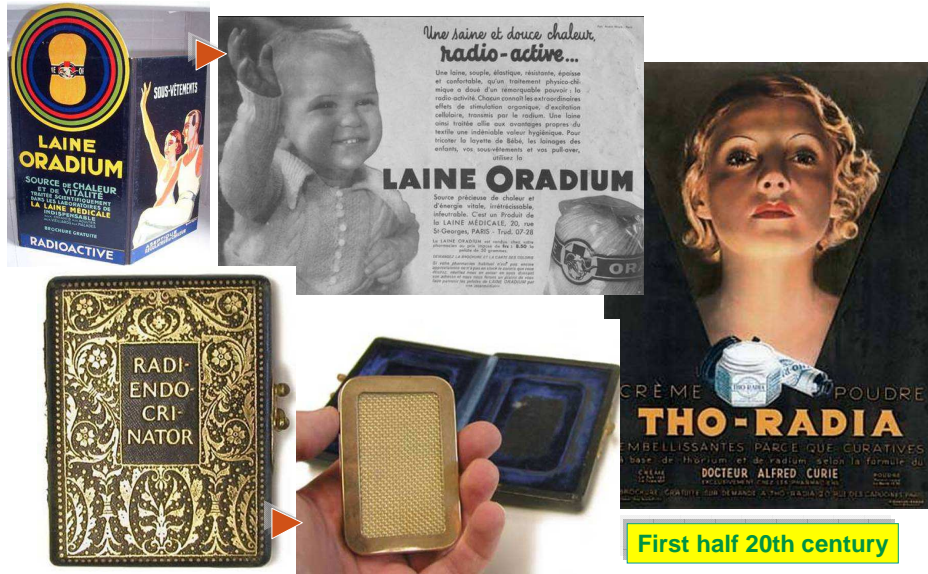
Founded
1978



Founded 1989



New => Topical, cosmetic-therapeutic applications



... And "internal" therapeutic applications



Clear signs of damage from overexposure => still assumption of safety at "low" doses



Measure radioactivity of Uranium and other ores with Gilbert Electroscop, just like real scientists.



Thrilling to watch! Gilbert Spinthariscopes shows you actual Atomic disintegration of radioactive material!



Prospect for Uranium and other radioactive Ores! Gilbert Geiger-Mueller Counter may win you \$10,000 Govt. bonus!

Decade 1950

Massive doses => Syndrome of the irradiated



At lower doses, mechanisms of effects unidentified => Safe

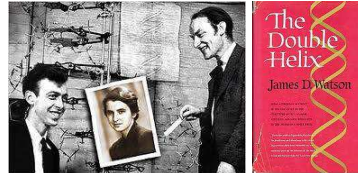


Thrilling to watch! Gilbert Spinthariscopes shows you actual Atomic disintegration of radioactive material!

RP: A recent discipline



1953: Watson & Crick (+ Rosalind Franklin) describe the structure of DNA (*The Double Helix*, Nobel Prize 1962) ► Mechanism => New paradigm in RP ► Best protection against long-term effects (although much remains unknown)



1998: ICNIRP sets Reference Levels for PR against immediate harmful effects of strong NIR ► Mechanisms for weak NIR unknown => effects of chronic exposure to weak NIR not considered => No RP against potential long-term effects

"Our current knowledge on the effects of NIR corresponds to that existing 70 years ago about the effects of IR"

SEPR course on "NIR in Medicine" (Málaga 2010)



Francisco Fernández Moreno, Advisor of CSN the (Nuclear Safety Council), Professor (UAB) y Academician

Weak (sub-threshold) NIR: Some evidence on short- middle-term effects

- Potentially deleterious

- – Semicircular lipotrophy (ELF) Human Stem cells
- Electrohypersensitivity (ELF, RF)
- Neurostimulation (RF)
- Brain blood flow (RF)

Not today
May be next time

- Therapeutic

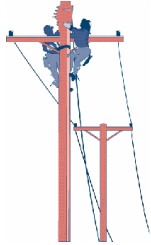
- Neurostimulation (ELF)
- – Oncostasis (RF) H. Neuroblastoma & Hepatocarcinoma
- – Tissue repair (ELF, RF) Human Stem cells

ELF (50 Hz): Long-term effects

Epidemiology of cancer and other diseases

Relevant effects at $f = 50$ Hz

Human cancer



- Leukemia and other cancers linked to occupational exposure to EMF (several studies)
- Childhood leukemia and residential exposure: $B \geq 0.4 \mu\text{T}$ (several studies)



Occupational exposure to 50 Hz (NR = $500 \mu\text{T}$)

Epidemiology: There is limited evidence of increased cancer risk in certain occupations with relatively high exposure

- Ahlbom et al., 2001. Review of the epidemiologic literature on EMF and Health. Environ Health Perspect 109:911–933.
- Johansen C. 2004. EMF and health effects—Epidemiologic studies of cancer, diseases of the central nervous system and arrhythmia-related heart disease. Scand J Work Environ Health 30:1–30.
- Feychting & Forssen. 2006. EMF and female breast cancer. Cancer Causes Control 17:553–558.
- Kheifets et al., 2008. Occupational EMF and leukemia and brain cancer: An update to two meta-analyses. J Occup Environ Med 50:677–688.
- Kheifets et al., 2009. Future needs of occupational epidemiology of extremely low frequency electric and magnetic fields: Review and recommendations. Occup Environ Med 66:72–80.

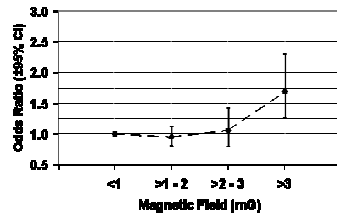
To be considered:

- Suboptimal metrology in some studies
- Potential confounders: Other carcinogens in the working environment?

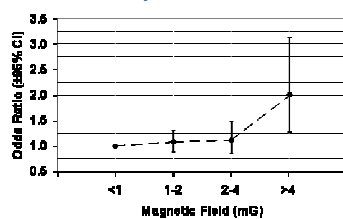


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ICNIRP'08: Risk Factors for Childhood Leukemia *RPD vol. 132/issue 2*



ICNIRP/WHO/BfS Workshop, Berlin 5-7 May 2008.

"Epidemiological studies of exposure to NIR in the ELF range (50/60Hz) have **consistently** shown an increased risk of leukemia at MF intensities from $0.3-0.4 \mu\text{T}$. There is no biological explanation for this effect"



ICNIRP maintains its RL of $B = 500 \mu\text{T}$ (occupational) and $B = 100 \mu\text{T}$ (public)

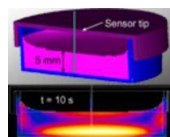
ELF experimental: Cancer

ICNIRP: “There is no biological explanation for such effect”=> **No biological phenomena** potentially involved in carcinogenic processes (proliferation, differentiation, apoptosis, tumoral markers expression...) **have been found** to be influenced by exposure to weak (sub-threshold) low frequency fields

Really?

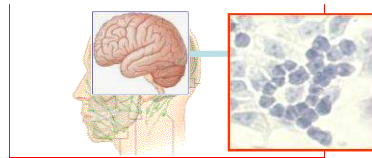
ELF: MECHANISMS

Experimental

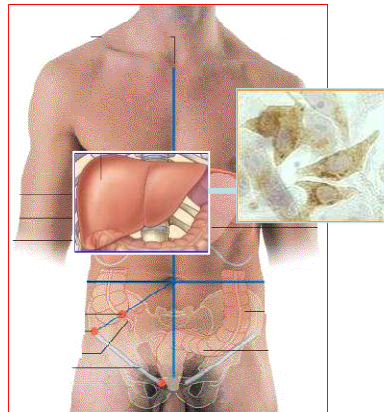


In vitro

Cellular models



A. Human cancer cells from neural origin (Neuroblastoma NB69)

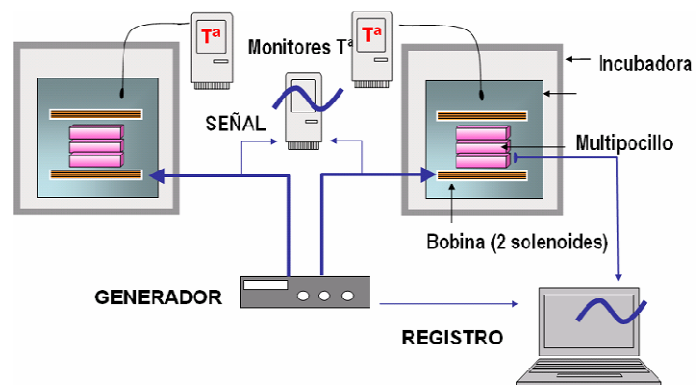


B. Human cells from liver cancer (Hepatocarcinoma HepG2)

- Are they responsive to weak (subthreshold) EM stimuli?
- If they are, how and through which mechanisms they respond? (characterization of the model and of its response)

Assays of sensitivity to ELF

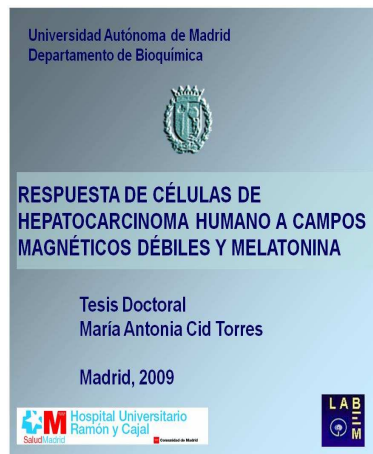
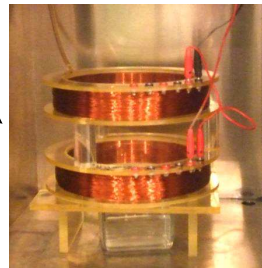
Exposure equipment: Double (experimental/control)



Assays of sensitivity to ELF

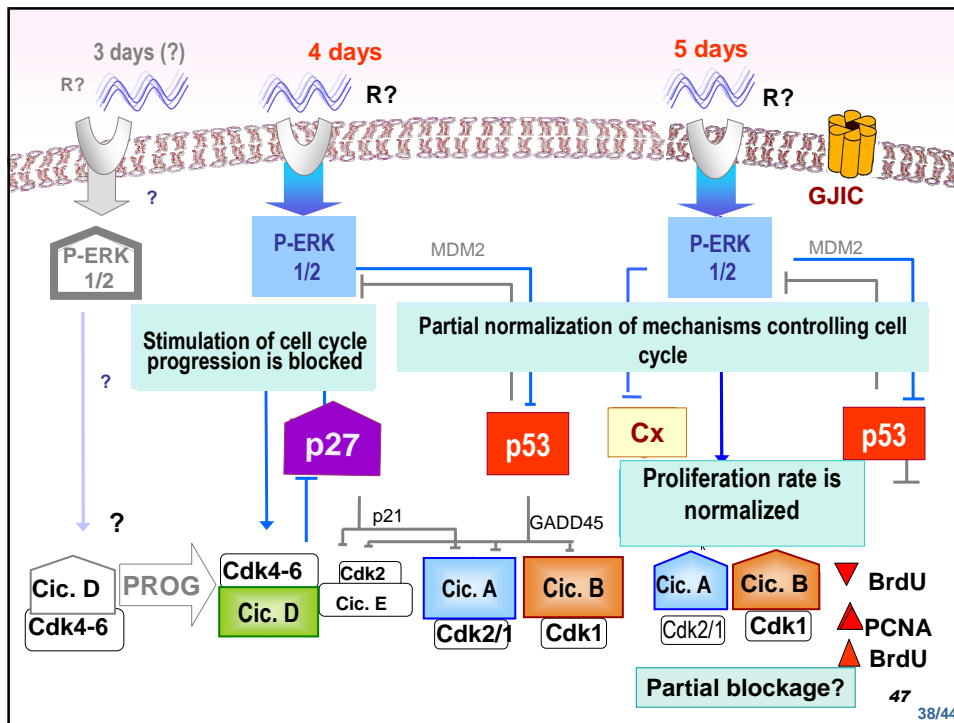


- ELF parameters:
50 Hz, $B \leq 100 \mu\text{T}$ (RL public)
- Exposure interval:
24 - 42 h
- Exposure cycle:
Intermittent; 3 h On/ 3 h Off



Our research group, 2009 – 2012

- Two Doctoral Thesis
- Five papers on ELF mechanisms



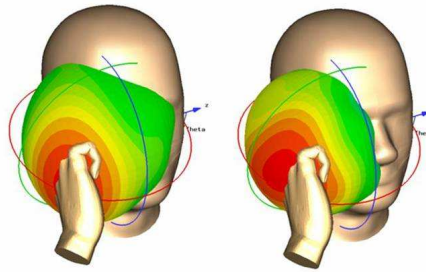
IONIZING RADIATION

vs NON-IONIZING



➤ **Ionizing radiation (RI)** has enough energy to remove an electron from an atom, producing an ion. Direct genotoxic action (40%) or indirect (free radical: 60%)

➤ **Non-Ionizing radiation (NIR)** lacks the energy to ionize: No direct genotoxicity. But "indirect" effects: on FR, DNA repair, gene expression... plus others, non genotoxic but potentially involved in cancer promotion/progression. **Several published and ongoing studies.**



RF: Short-term human effects

Many studies, mostly on M.Ph. Sometimes mixed results. The strongest data (replicated independently) ---➔

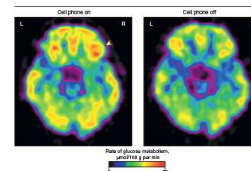
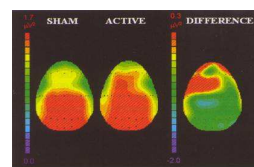
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Summary: Short-term effects (30–50 m)

1. Neurostimulator (ipsilateral)
 - Enhances α EEG signal
 - Changes (Δ / ∇) in glucose metabolism (=MTS)
2. Blood flow increase (ipsilateral)
3. Cognitive (changes in reaction time)
- **Subthermal**

- Reversible in the short-term
- “Not demonstrative of short-term damage” (ICNIRP)
- **What about in the middle / long-term?**



RF-MW: Long-term effects

Epidemiology (reproduction & cancer)



Epi. occupational exposure to RF-MW subthreshold

- Cancer (leukemia, lymphoma) mortality in Belgian workers operating military radars (2009)
- Fetal loss and low neonatal weight in female physiotherapists applying MW and SW during the first months of pregnancy (USA, Israel and other countries: 1998 and later)
- Low fertility in sailors of the Danish navy exposed to radar emissions (2007)
- Low fertility in the crew of fast boats of the Swedish navy exposed to radar emissions (2011)
- To be considered:
 - In general, insufficient or inexistent metrology
 - Biases: Statistical or selection of cases/controls?
 - Confounding factors: Other harmful agents present in the occupational environment?

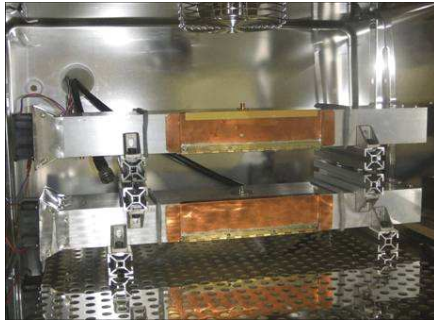
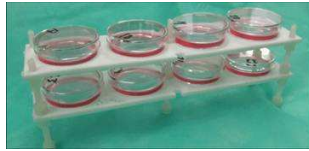
Epi. studies, general public,
mobile phones & cancer
INTERPHONE and others



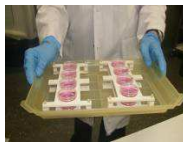
Oncological effects of weak RF.
Experimental in vitro

- Several studies (50%) have reported evidence of cellular response to different RF signals at doses \leq ICNIRP limits.
- The underlying mechanism presently investigated would involve biophysic phenomena more complex than the thermal ones, the only considered by ICNIRP for protection purposes.

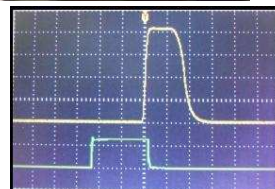
RF exposure in vitro: Methodology



RF in vitro: Methodology



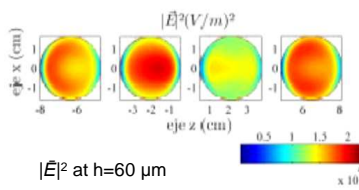
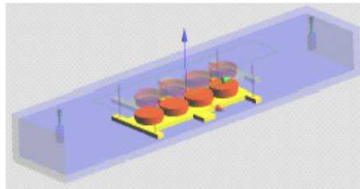
RF: 2.2 GHz, pulse modulated
Peak field (CW) :
 $E = 3,77 \text{ kV/m}$
 $S = 13,15 \text{ kW/m}^2$
Pulse duration: $5 \mu\text{s}$
Repetition rate: 100 pps
Exposure interval: 24 h



Metrology (Numerical “dosimetry”)



•Telecom. Sch., Polytechnic Univ. Madrid
•Fac. Phys. Sci. Granada Univ.



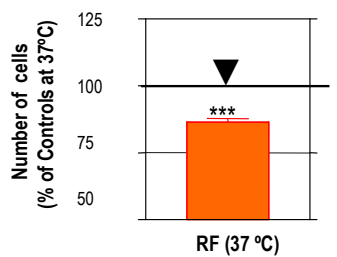
- FDTD software (SEMCAD X)
- Heterogeneous distribution of field
- Average SAR for CW: 44.8 W/Kg
- Short pulse modulation: 5 μs pulses at 100 pps. + heat dissipation \Rightarrow No significant thermal increase after 24h exposure ($\Delta T < 0,001 \text{ } ^\circ\text{C}$)



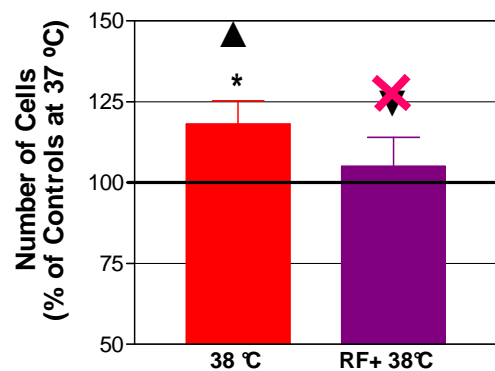
Subthermal (microthermal) exposure?...

Basic positive control: Mild hyperthermia (+1 $^\circ\text{C}$)

Growth response of NB69 after 24 h exposure

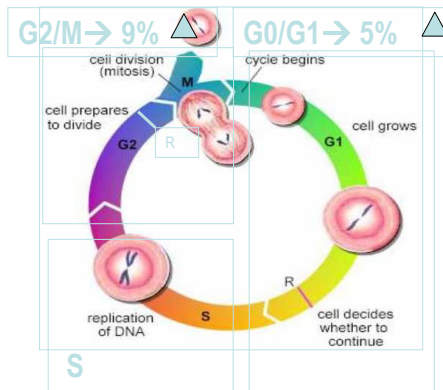


N = 8 indep. reps

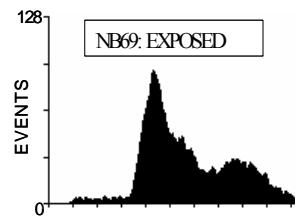
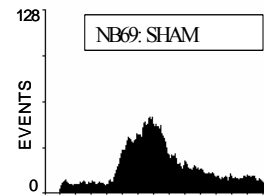


N = 5 indep. reps

Changes in the cell cycle (Flow Cytometry, N = 8 independent repeats)



NB69: PARTIAL BLOCKING (14% cells)



CONCLUSION

1. RF exposure induced a significant reduction in cancer cell growth
2. The antiproliferative effect is associated to an arrest of the cell cycle at phases G₂/M y/o G₀/G₁ => Cytostatic (Oncostatic?) action
3. As a whole, the results indicate that the effect is of EM nature, rather than thermal

In fact, cytostatic/oncostatic action (NS and/or liver) of subthermal RF E/MF (SAR 100 – 1000 fold < M.Ph) has been repeatedly observed both in cells as in humans => Some therapies emerging

CRET:

- Hernández-Bule et al., 2004
- 2007
- 2010
- 2011
- 2012 (prep)

TheraBionic:

- Barbault et al., 2009
- Costa et al., 2011
- Zimmerman et al., 2012



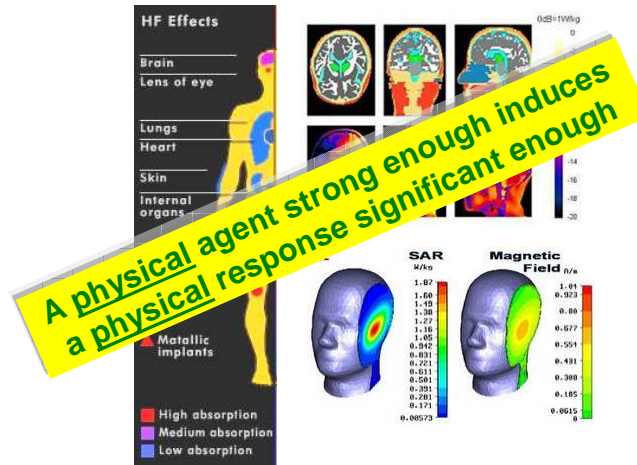
Mechanisms: RF in vitro

REPORTED EFFECTS ON:

- Genotoxicity (ADN)
- Cell function: Proliferation, Apoptosis, Gene and protein expression → Mixed results and effects depending on physical and/or biological parameters
- Recent results: Activation of cellular stress responses (hsp27 / hsp70/ ROS/ TAC/ MAPK p38)

“Signal transduction” hypothesis: Some cellular/molecular structures could identify specific RF signals (considering LF modulation) as a physical or chemical stimulus. Those structures would act as physical ↔ chemical transducers.

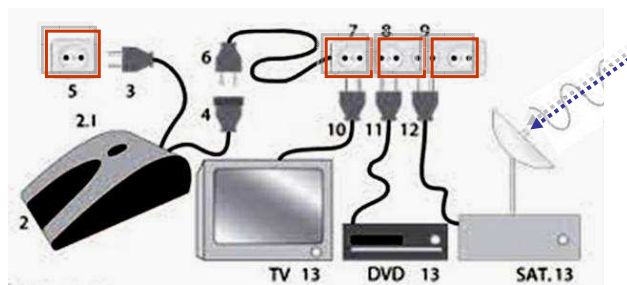
RP against thermal effects of RF-MW: thermal SAR, $\Delta T > 1^\circ\text{C}$



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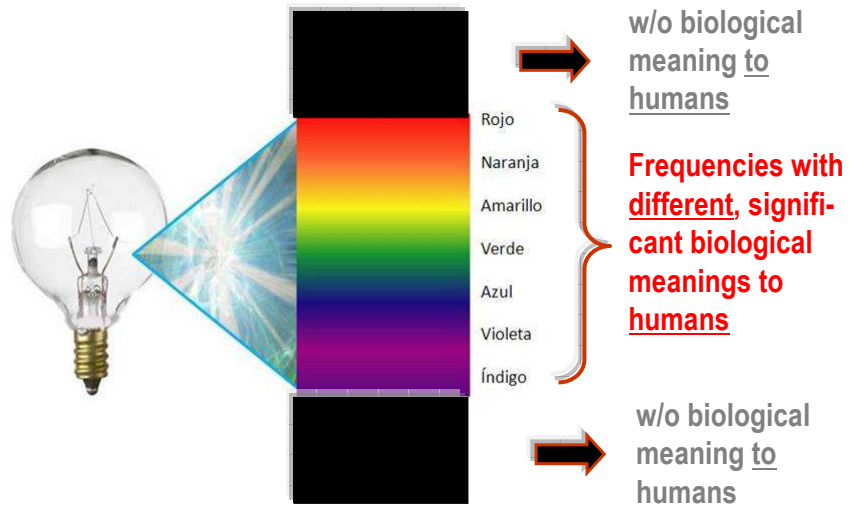
However, in the case of subthermal effects the amount of absorbed energy (SAR) should not be the main parameter to be considered. A weak signal carrying a “biological” message can induce significant bioeffects. The energy for the response (FR, gene activation, cytoproliferation...) is not supplied by the signal...

... but by the metabolism of the biosystem (organism)

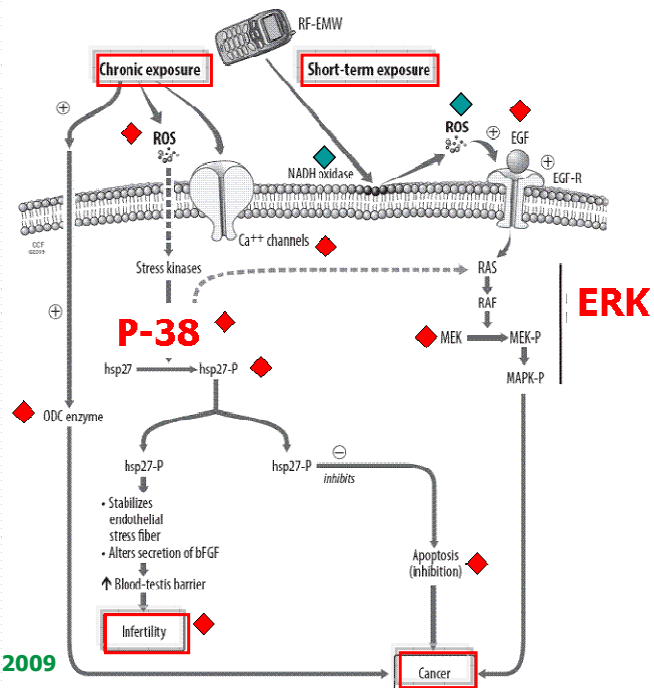
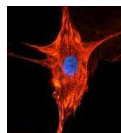


IN FACT...

A weak signal with a biological meaning can induce significant bioeffects



HYPOTHESIS



Review, Desai y col., 2009



Conclusions & ending comment 1

- There are indications (not considered “evidence of damage” so far) that exposure to sub-threshold doses of NIR can induce short-and long-term effects in humans.
- => New studies are needed to: 1) definitively rule out the possibility of deleterious, sub-threshold effects, or 2) characterize the conditions for damage and sensitivity.
- It is not possible (time and effort) to investigate every specific source (MRI, Radar, MW, M.Ph4D, Wi-Fi, Wi-Max...) and exposure condition for general public and workers.

Thus...

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Conclusions & ending comment 2

- It is crucial to extend our knowledge on the cellular-molecular response => **Identify mechanisms**
- **That would allow:**
 1. Re-design, re-address and interpret human studies
 2. Develop efficient, preventive strategies for protection of the public and the workers
 3. Improve the present diagnostic and therapeutic systems based on NI radiation, and develop new, non-invasive therapies, that are more efficacious and free from unwanted side effects.

Meanwhile, prevention and information...

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¡Muchas @racias!

Thank you so much!