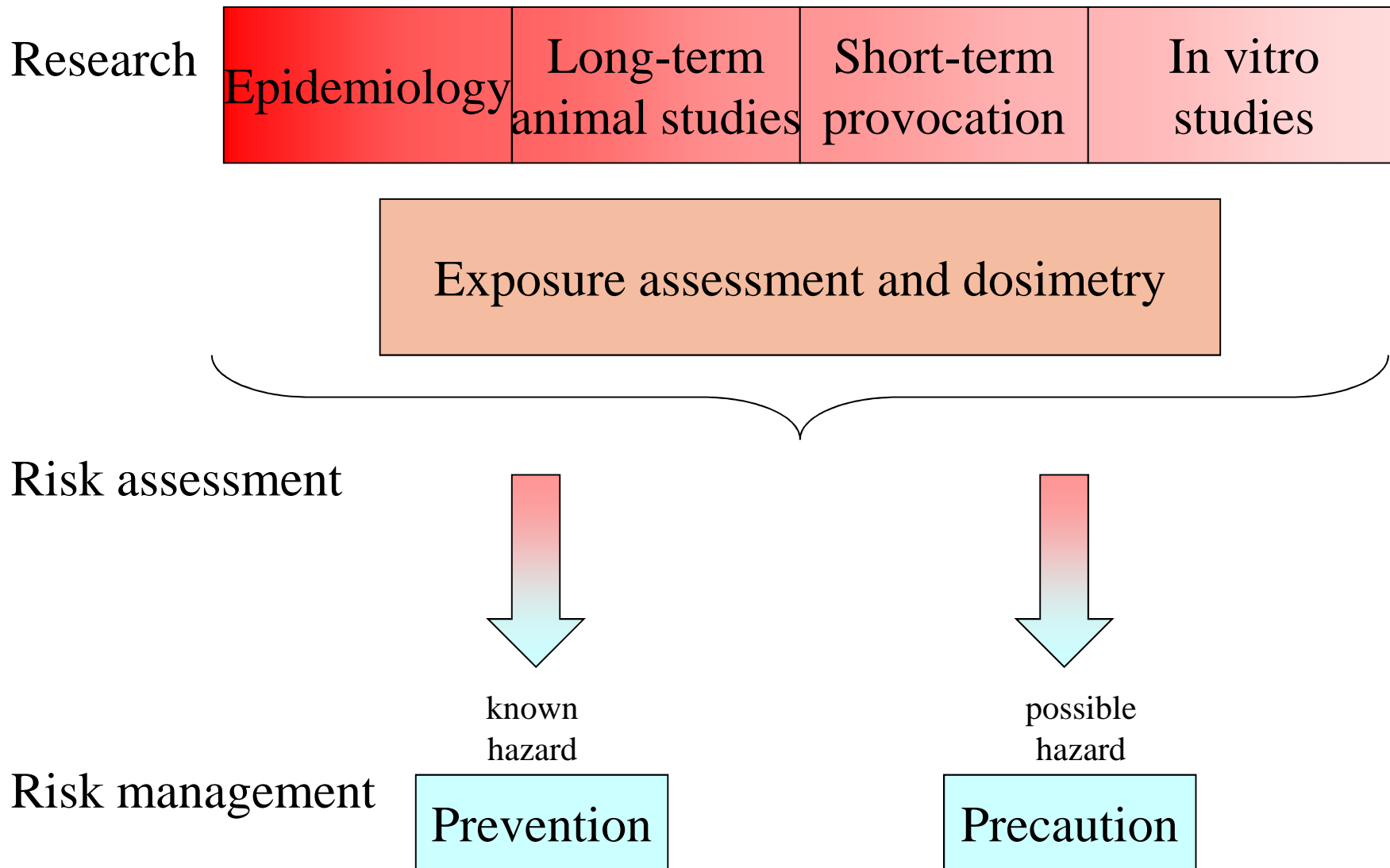


Problems of the design and analysis of animal experiments on chronic effects of EMF

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IARC Classification

Category of evidence	In humans	In animals
Sufficient evidence of carcinogenicity	A causal relationship has been established between exposure to the agent, mixture, or exposure circumstances and human cancer. That is, <u>a positive relationship has been observed between the exposure and cancer in studies in which chance, bias, and confounding could be ruled out with reasonable confidence.</u>	A causal relationship has been established between the agent or mixture and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in <u>a) two or more species of animals or b) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.</u>
Limited evidence of carcinogenicity	A positive association has been observed between exposure to the agent, mixture, or exposure circumstance and cancer for which a causal interpretation is considered to be credible, but chance, bias, or confounding could not be ruled out with reasonable confidence.	The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, for example, a) the evidence of carcinogenicity is restricted to a single experiment; b) there are unresolved questions regarding the adequacy of the design, conduct, or interpretation of the study; or c) the agent or mixture increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential, or of certain neoplasms that may occur spontaneously in high incidences in certain strains.
Insufficient evidence of carcinogenicity	The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, or no data on cancer in humans are available.	The studies cannot be interpreted showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations, or no data on cancer in experimental animals are available.
Evidence suggesting lack of carcinogenicity	There are several adequate studies covering the full range of levels of exposure that human beings are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent, mixture, or exposure circumstance and any studied cancer at any observed level of exposure.	Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent or mixture is not carcinogenic.

Evidence for animal carcinogenicity before the MP era

Study	Species	Induction	Experimental conditions	n	SAR [W/kg]	Pre-induction exposure	Exposure Condition					
							Frequency	ELF modulation	Pulsed	near/far-field	Duration/day sessions/week	Overall duration
Chou et al. 1992	Sprague-Dawley rats	none	sham	100	-	-	2.45 GHz	8 Hz	10 µs	far	21.5 h	24 months
			exposed	100	0.15-0.4							
Szmigielski et al. 1982	C3H/HeA mice	(milk-borne virus)	cage controls	40	-	no	2.45 GHz	no	no	far	2 h	12 months
			sham	40	-							
			confinement	40	-							
			exposed 1	40	2-3							
			exposed 2	40	6-8							
	Balb/c mice	chemical induction (B(a)P)	cage controls	40	-	-	2.45 GHz	no	no	far	2 h	12 months
			sham	40	-	-						
			confinement 1	40	-	1 month						
			confinement 2	40	-	3 months						
			exposed 1	40	2-3	1 month						
Balb/c mice	chemical induction (B(a)P)	cage controls	40	-	no	2.45 GHz	no	no	far	2 h	12 months	
		sham	40	-								
		confinement	40	-								
		exposed 1	40	2-3								
		exposed 2	40	6-8								
Szudzinski et al. 1982	Balb/c mice	chemical induction (B(a)P)	sham	100	-	no	2.45 GHz	no	no	far	2 h	6 months
			exposed 1	100	2							
			exposed 2	100	6							
	Balb/c mice	chemical induction (B(a)P)	sham	100	-	-	2.45 GHz	no	no	far	2 h	7 to 9 months
			exposed 1	100	4	1 month						
			exposed 2	100	4	2 months						
Santini et al. 1988	black C57/6J mice	tumour cell implantation	sham	15	-	15 days	2.45 GHz	25 Hz	10 µs	far	2.5 h	6 weeks
			CW	15	1.2							
			PW	15	1.2							

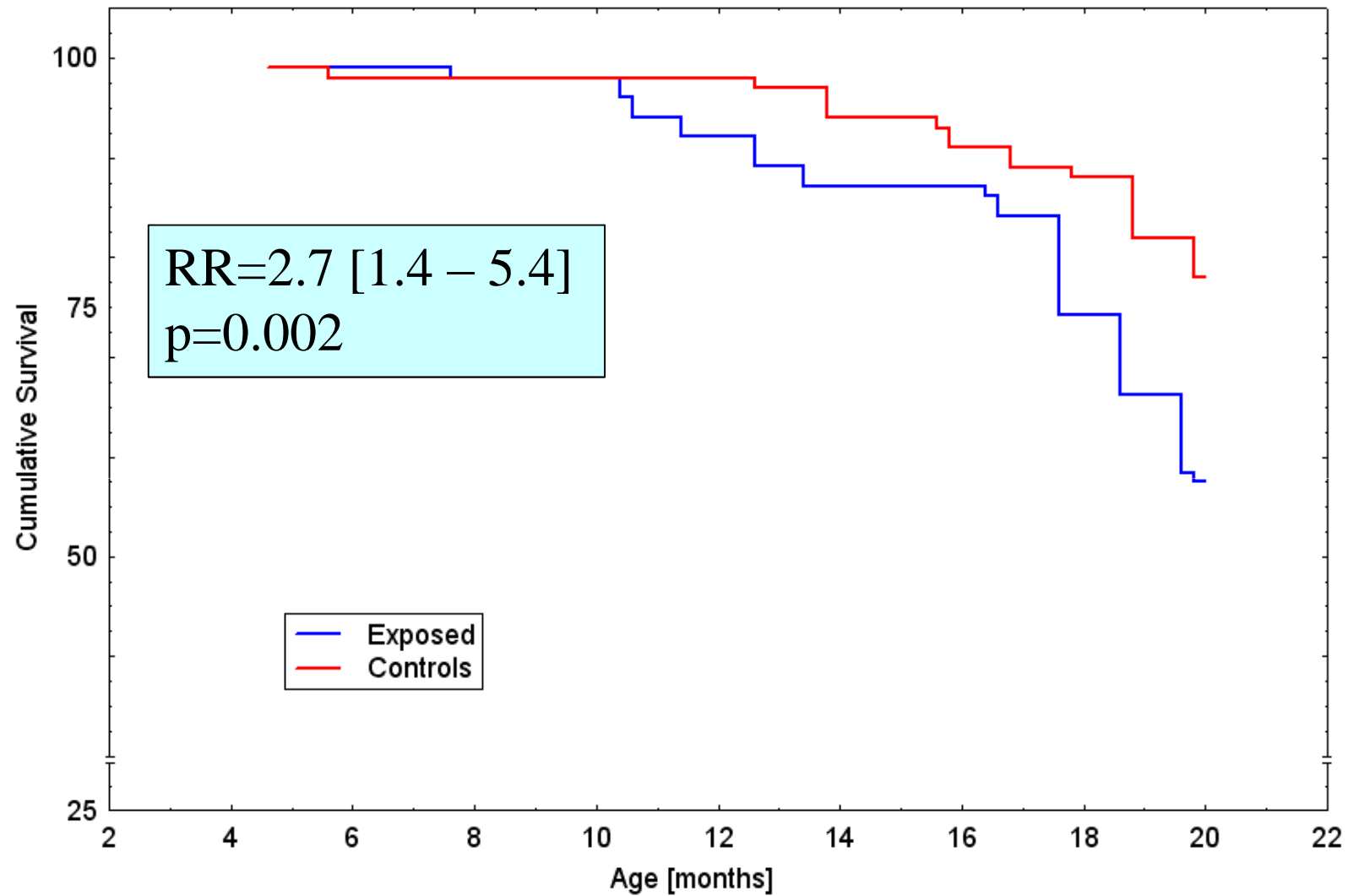
Evidence for animal carcinogenicity before the MP era

- The evidence for carcinogenicity in animals was at that time sufficient
 - Increased incidence of malignancies in two species (rats and two strains of mice)
 - Evidence from two different labs
 - Evidence from three different protocols

Due to the fact that not even limited evidence for carcinogenicity in humans was available at that time (due to the lack of conclusive studies) no classification according to the IARC procedure was possible.

The Repacholi et al. (1997) study

- Because of the intriguing findings in animal assays that were at odds with the general believe that no adverse effects should occur at non-thermal levels a decisive experiment was planned
- A **sensitive mouse model** was used (E μ -Pim1)
- Sample size (n=100) was chosen to guarantee sufficient **power to detect a 2-fold increased risk**
- Instead of 2.45 GHz, 900 MHz and 217 Hz, 0.6 ms pulse-repetition rate was chosen to **simulate GSM signals**
- Duration of exposure was set to **2 times ½ h per day 7 days/week, for up to 18 months**



Comparison with ‘replication’ trials

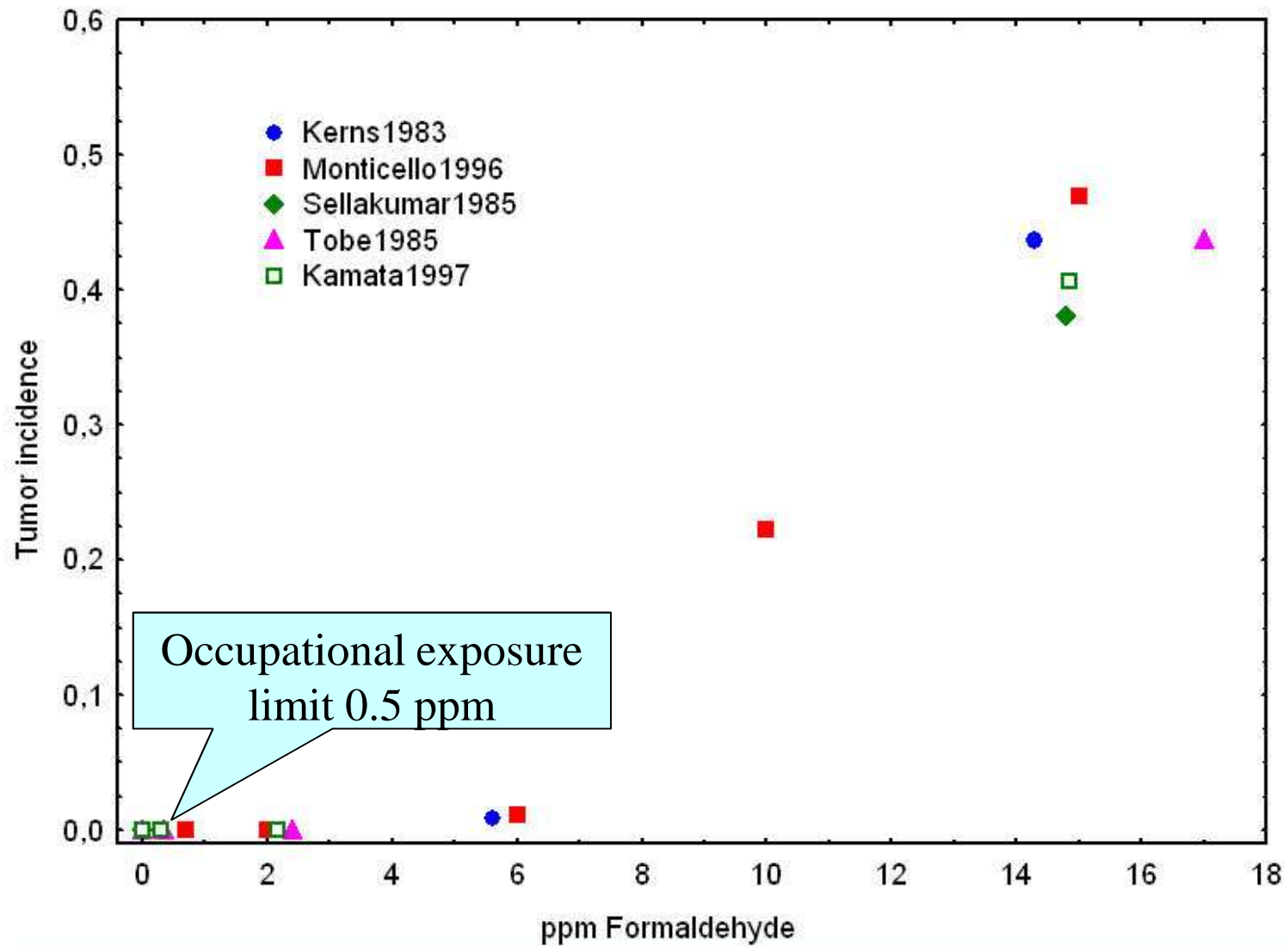
Exposure condition	Repacholi et al. 1997	Utteridge et al. 2002	Oberto et al. 2007
Cage controls	-	(74%)	52%
Sham	22%	44%	44%
0.25 W/kg		43%	
0.5 W/kg			36%
~1 W/kg	43%	40%	60%
2 W/kg		45%	
4 W/kg		42%	40%

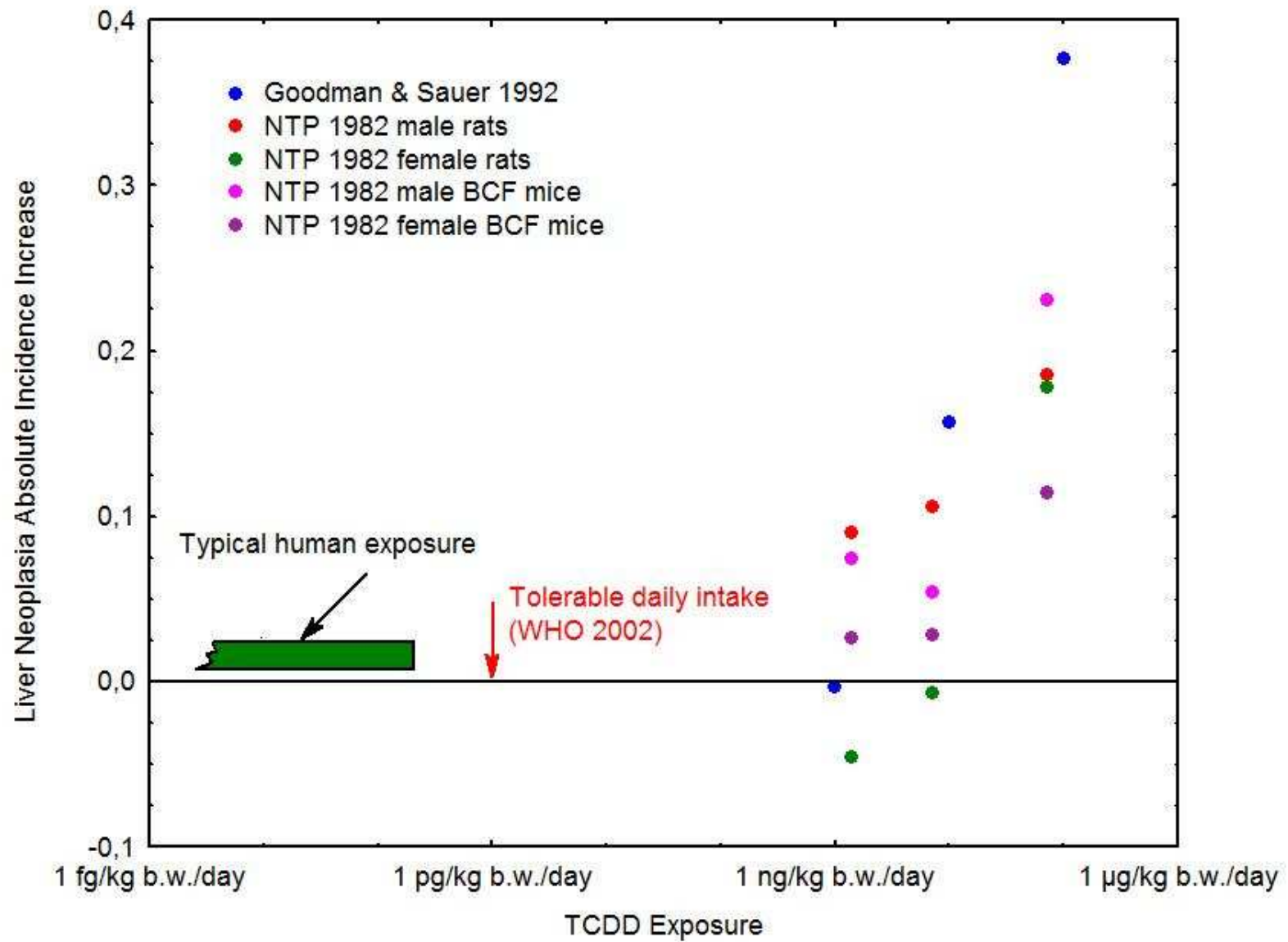
Gold standard of lifetime animal carcinogenicity assays

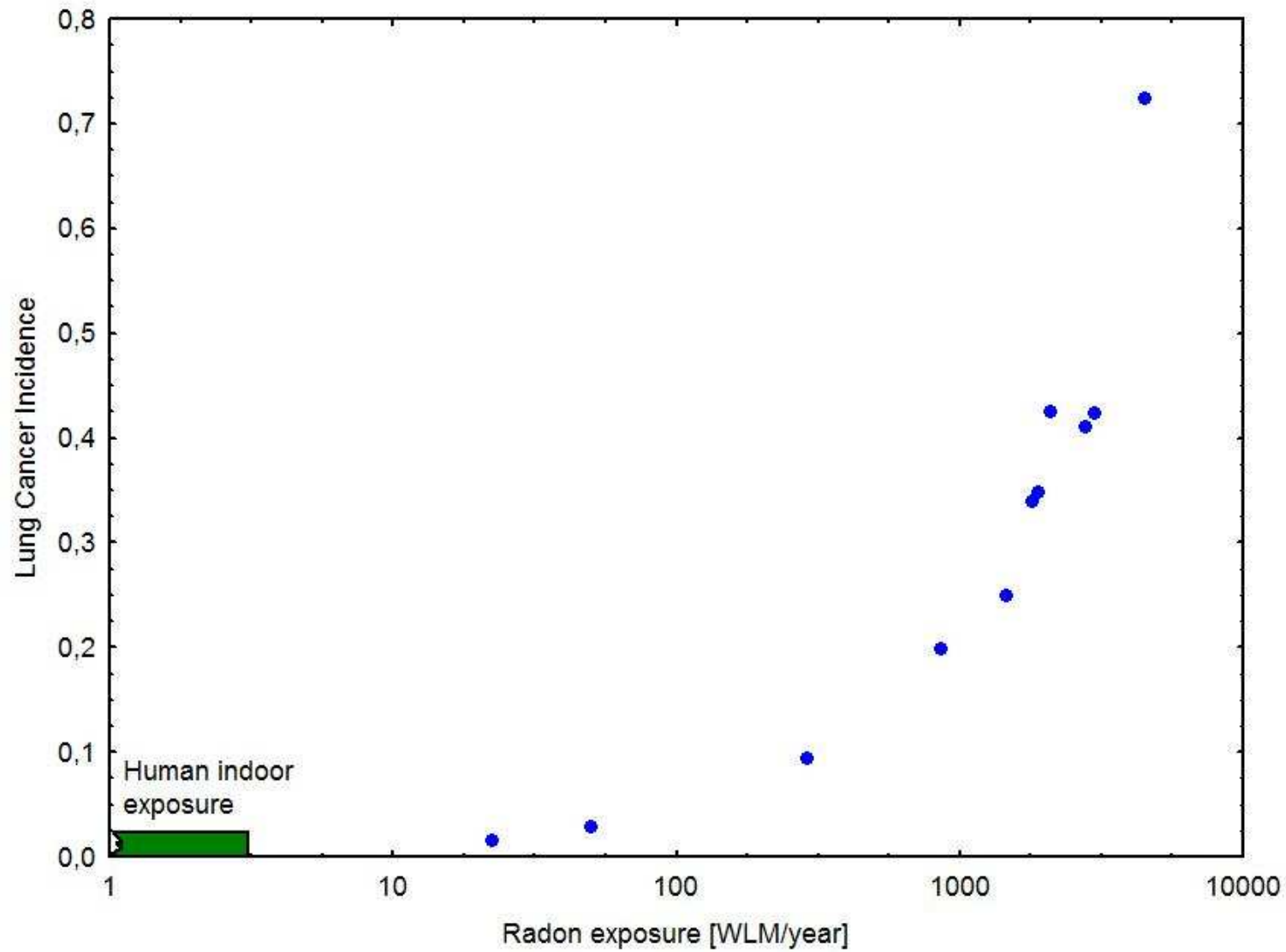
- **Strains:** two rodent strains, both sexes (typical: F344 rats and B6C3F1 mice), n ~50 per strain/sex
- **Exposure:** 2 to 3 doses starting with the MTD (maximum tolerated dose) and geometrically lower doses (factors 2 to 10) and a control group
- **Duration:** starting with ~8 weeks for 94 weeks
- **Conditions:** SPF housing, specific exposure chambers if necessary

Problems for the study of EMF exposures

- For virtually none of the established chemical carcinogens an increase above background can be demonstrated in animal assays at levels found in human environments
- E.g. instead of 100 animals per dose about 13000 animals would be needed to demonstrate a rate increase for formaldehyde at human exposure levels
- Therefore, because RF EMF cannot be of distinctly higher carcinogenic potential than typical chemical carcinogens, special considerations are necessary







Design considerations for EMF studies

Procedure chosen for chemical agents not possible

- At human exposure levels it is unlikely to detect an increased risk in the standard animal carcinogenicity assay
- An increase of exposure intensity as chosen in chemical carcinogenicity assays is not possible because of induction of significant heating that would interfere with malignant transformation and development

Design considerations for EMF studies

Increase power

- In order to increase the power of the assay the background incidence of malignancies must be increased
 - by implantation of tumor cells
 - by pre- or concomitant exposure to a known carcinogen
 - by using strains with increased incidence (e.g. transgenic animals, endogenous viral oncogens)
- Megaexperiments with several 1000 animals are possible, but only in a few labs

Design considerations for EMF studies

Consider weakness of the effect

- Due to the low carcinogenic potential at human exposure levels only assays with certain characteristics are suitable
 - late onset of malignancies
 - slow increase of incidence
 - background cumulative incidence between 20-40%

Design considerations for EMF studies

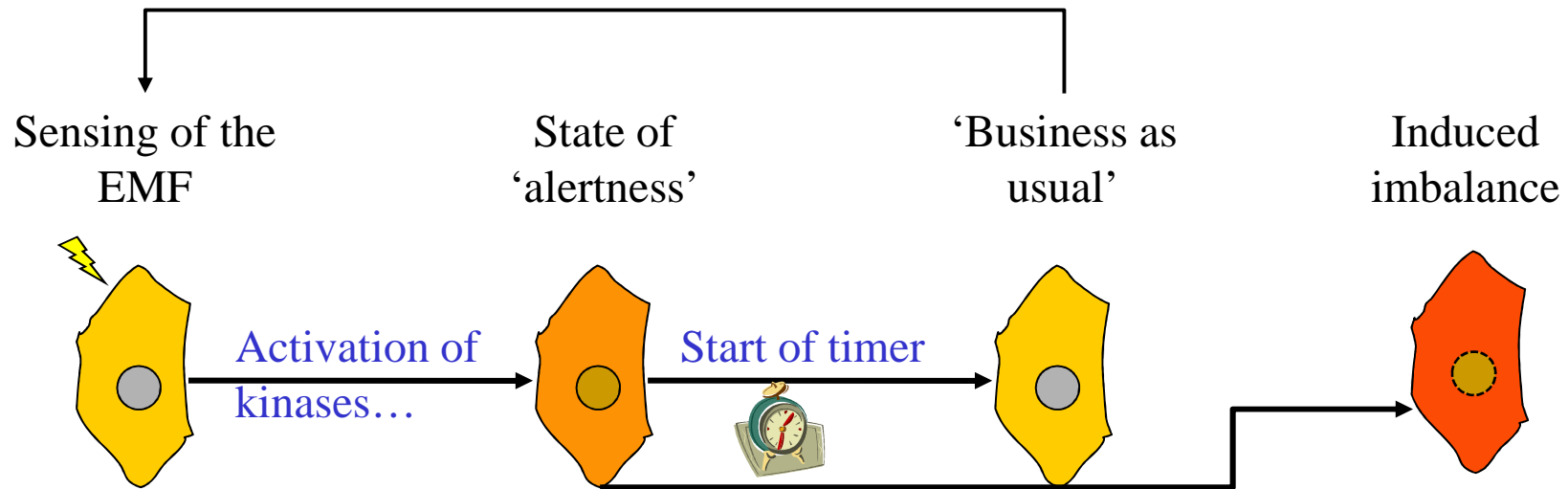
Take care of confounding conditions

- Cancer evolves from an imbalance between the mechanisms that control proliferation and apoptosis
- Therefore, experimental conditions must avoid interference with these mechanisms
- Examples of such conditions are
 - stress from isolation, immobilization etc.
 - infection
 - inadequate nutrition

Design considerations for EMF studies

Carefully consider exposure conditions

- Older experiments have been criticized for their broad variation of exposure
- But: because we have no sound model of an interaction mechanism at low exposure levels, broad variation is not so bad!
- Avoid artificial constancy of exposure while maintaining a sufficient gradient of exposures across dose groups
- If exposure has low temporal variation choose intermittent exposure
- Don't jump to conclusions: do not assume that the effect will increase with duration or intensity of exposure



Could be an all-or-nothing process!
Intensity might only be associated with the number (!) of cells activated

Likely depends on cell type!
Stem cells will only respond when not in G0.
Response is likely unspecific i.e. not confined to EMF

If during state of alertness no damage is sensed the reaction is turned off.
This seems to happen after several minutes

In cells with defect timer or after repeated on/off of the alertness state a cell may become habitually imbalanced

Design considerations for EMF studies

Carefully consider exposure conditions

- Choice of the frequency of the carrier signal must be justified
- It has to be borne in mind that the distribution of the EMF within the organism will be distinctly different in humans as compared to rodents
- To have a similar pattern, scaling of frequencies should be considered
- This choice will depend on the assumptions about the basic interaction mechanism. If only the total absorbed energy counts, frequency is irrelevant.

Analysis of experiments

- For standard assays the primary endpoint should be **time to occurrence** of the neoplasm under study
- Secondary endpoints include all malignant and all benign neoplastic lesions, tumor volume, tumor multiplicity etc.
- Indicators such as body weight, body temperature etc. as well as blood and urine parameters may be obtained if this can be accomplished with minimal stress

Analysis of experiments

- Many studies used inappropriate statistical methods such as comparison of cumulative tumor incidences
- Non-parametric comparisons of life-tables such as the log-rank test and its extensions should be used
- Alternatively, more powerful methods are available if time to occurrence can be shown to follow a certain distribution such as the Weibull or Gompertz distribution
- If correction for confounders is to be applied, Cox' regression and similar methods are appropriate

Evidence to date

- About 30 long- and medium-term animal carcinogenicity studies have been published to date
- Only few fulfill the criteria mentioned above
- Many have insufficient power, most have a very steep gradient of incidence increase due to the induction method applied (sometimes all animals die within a few weeks!)

Summary

The large number of animal carcinogenicity studies **has not** increased our knowledge about a potential cancer risk of RF EMF. Risk assessment is left with almost nothing that can be used for this purpose because **most of the studies are inappropriate** and inconclusive.

It has to be stressed that animal experimentation in the case of EMF is **extremely difficult**. There is no other agent with comparable difficulties.

An international expert group should convene to provide solutions to these intriguing problems.