

Cytogenetic Studies In Radiofrequency Radiation Research

Past, Present and Future

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Genetic Damage Investigations

Most genotoxic agents are
CARCINOGENS

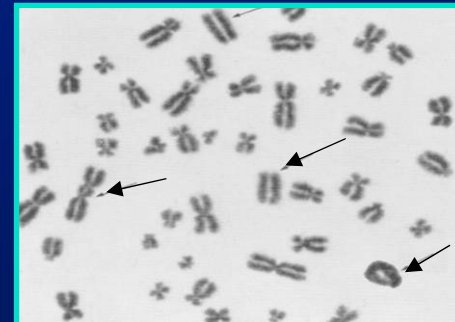
Non-genotoxic agents
which do NOT cause damage by themselves
can also contribute to **carcinogenesis**
by **enhancing** the damage induced by
known genotoxic agents

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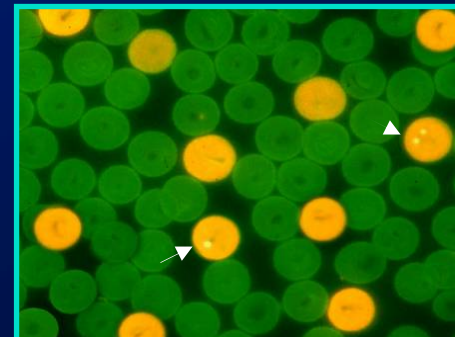
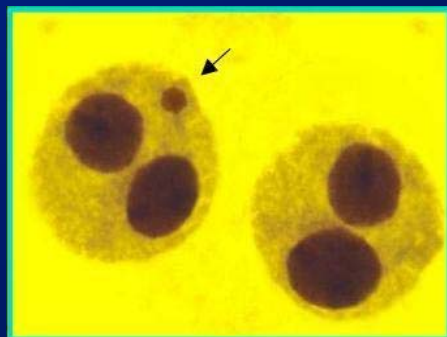
DNA Strand Breaks
SSB / DSB



Chromosomal Aberrations
CA



Micronuclei
MN



Sister Chromatid Exchanges
SCE



PAST

1990- 2003

“ Qualitative Assessment ”

Test System	Number of Studies Indicating Damage			Total
	Increase	No Increase	Inconclusive	
<u>DNA Strand Breaks</u>				
Whole-Body Exposure: Animals	4	1	0	5
<i>In Vitro</i> : Cultured Rodent Cells	0	3	0	3
<i>In Vitro</i> : Cultured Human Cells	0	2	1	3
<i>In Vitro</i> : Human Blood Lymphocytes	0	4	2	6
<u>CA, MN & SCEs</u>				
Whole-Body Exposure: Normal Animals	0	2	1	3
Whole-Body Exposure: Transgenic Animals	1	0	1	2
Whole-Body Exposure: Human	2	1	1	4
<i>In Vitro</i> : Cultured Rodent Cells	2	1	0	3
<i>In Vitro</i> : Human Blood Lymphocytes	3	11	4	18
<i>In Vitro</i> : RF alone (+/- Genotoxic Agents)	0	6	0	6
Total	12	31	10	53
	23%	58%	19%	
<i>In Vitro</i> : RFR +/- Genotoxic Agents	1	3	2	6
	17%	50%	33%	

Recommendation

International Collaborative study
Co-ordinated in 6 separate centers
Adequate statistical power

Guide-lines

RFR exposures in a single laboratory
SAR 1 – 5 W/kg
Validated dosimetry
Adequate temperature controls
Multiple genotoxicity end-points
Multiple cell types of human origin
Different genetic backgrounds

P R E S E N T

German Mobile Telecommunication Program

Funding

Federal Office for Radiation Protection

RFR-exposure Equipment & Dosimetry

IT'IS, Zürich

Donor Recruitment, Blood Sampling, Exposures & Analyses

INCOS & IMBEI, University of Mainz

Genotoxicity Evaluation

Dermatology Center, Buxtehude (near Hamburg)

University of Applied Science, Darmstadt (near Frankfurt)

Cytotest Cell Research GmbH, Roßdorf (near Gottingen)

'Blind' Study

Peripheral Blood Donors

10 males - between 50 - 60 yrs

10 males - <18 years

RFR-exposure G₁, S & G₂ phases of cell cycle

Frequency 1800 MHz

SAR 0.0, 0.2, 2 & 10 W/kg

Exposure 24 hours

Genotoxicity End-points

Alkaline Comet Assay 100 cells / donor

Chromosome Aberrations 1000 cells / donor

Micronuclei 2000 cells / donor

Sister Chromatid Exchanges 50 cells / donor

Project Duration 2.5 years

Meta-Analysis

“ Quantitative Assessment ”

Meta-Analysis

Utilizes several quantitative statistical methods
for large data review & analysis

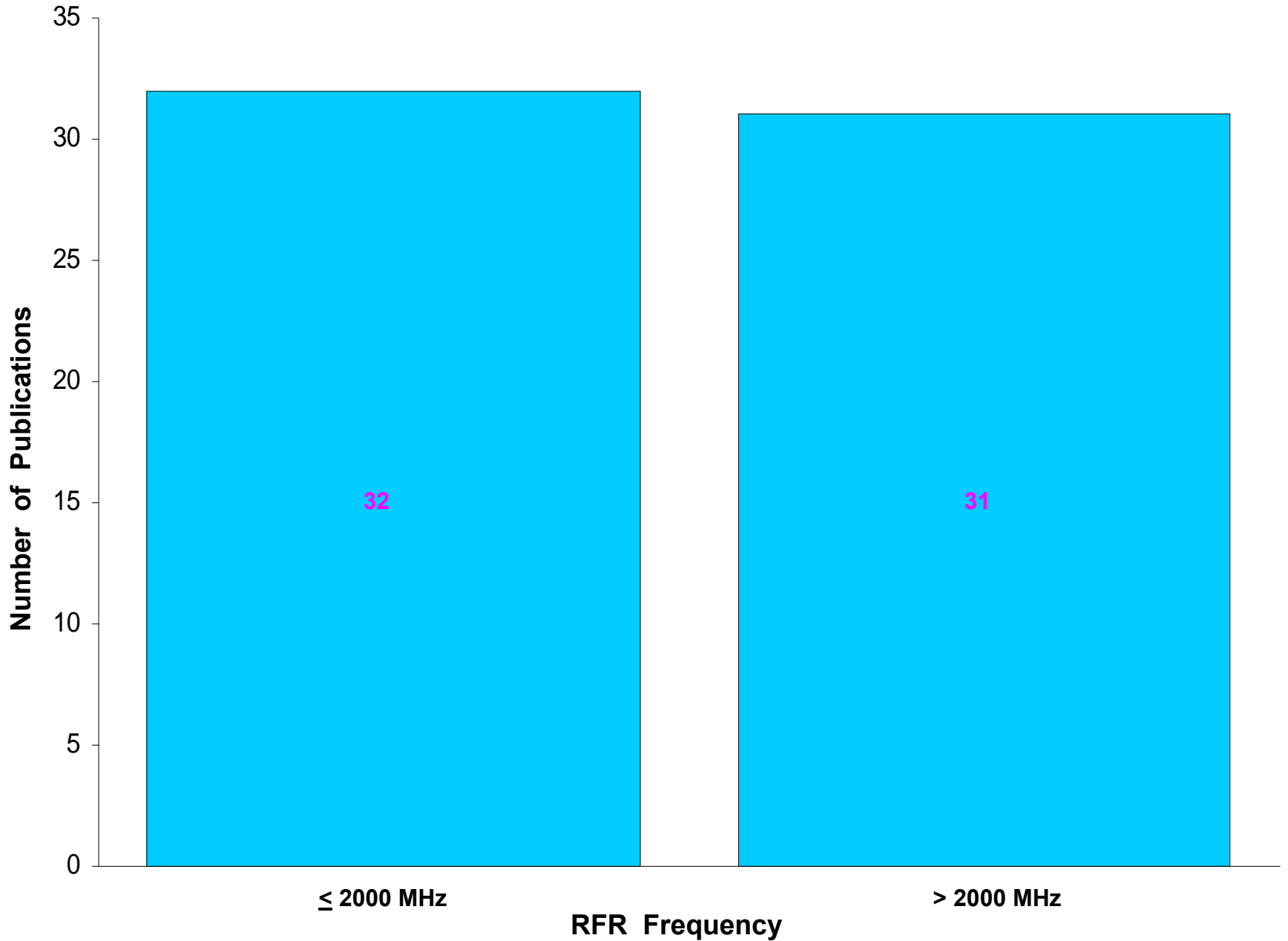
Widely used in biomedical research ESPECIALLY
when the outcomes in different investigations are
controversial

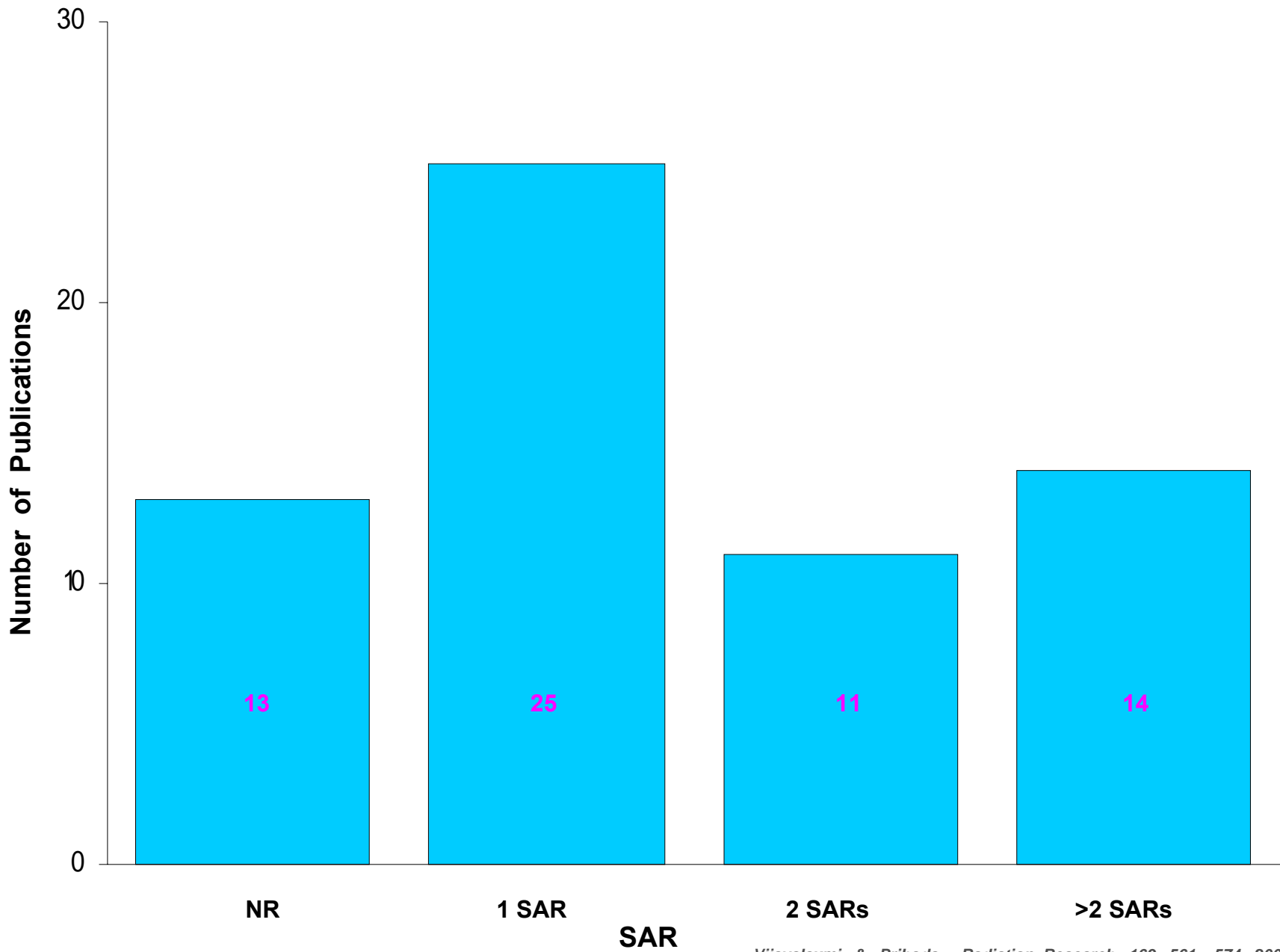
If considered separately, any one study may be
too small to arrive at a generalized / unequivocal
conclusion

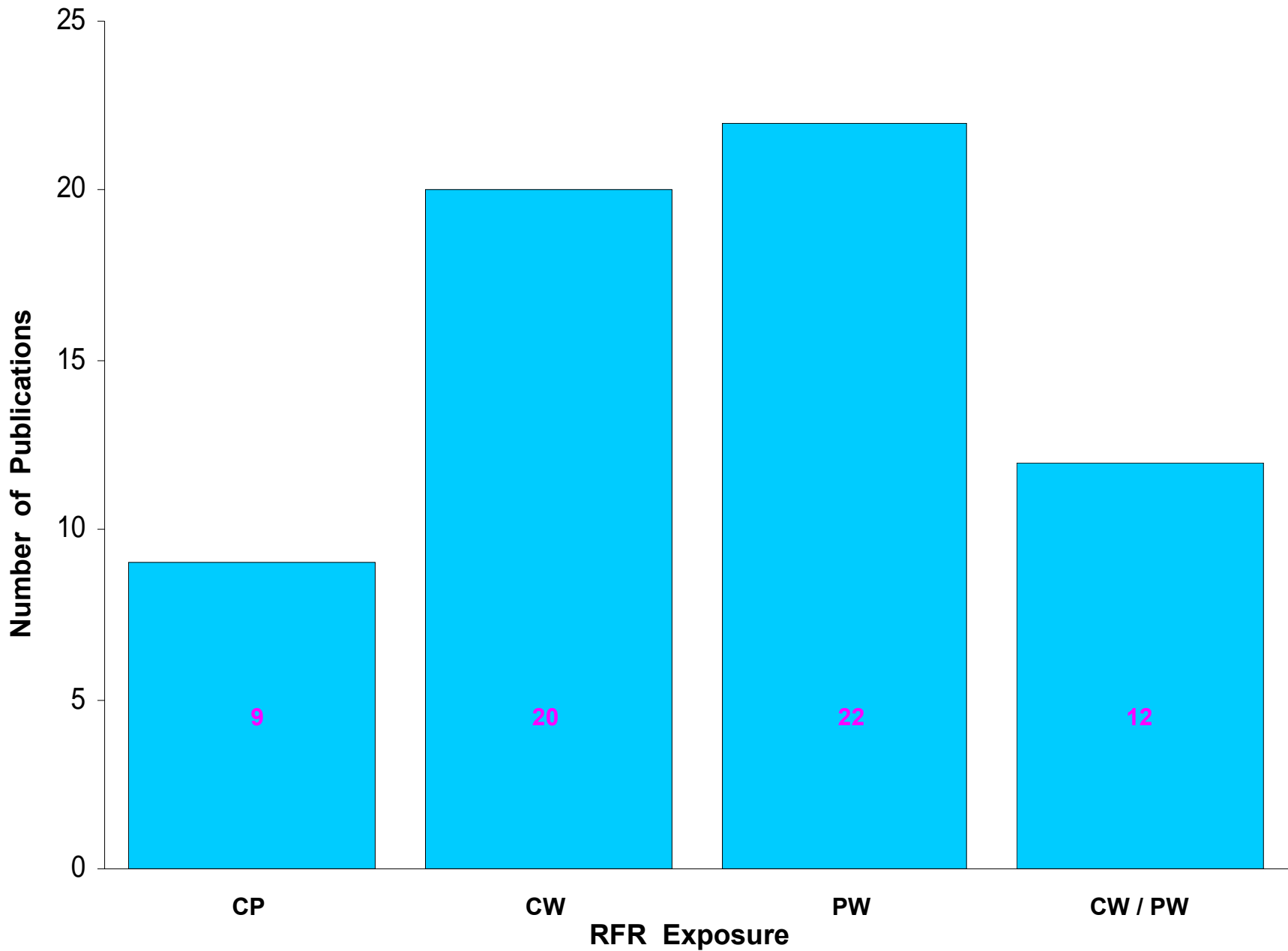
**ANALYSIS OF THE COMBINED DATA FROM
ALL RELATED STUDIES**
an attractive alternative to strengthen the evidence
from any individual study

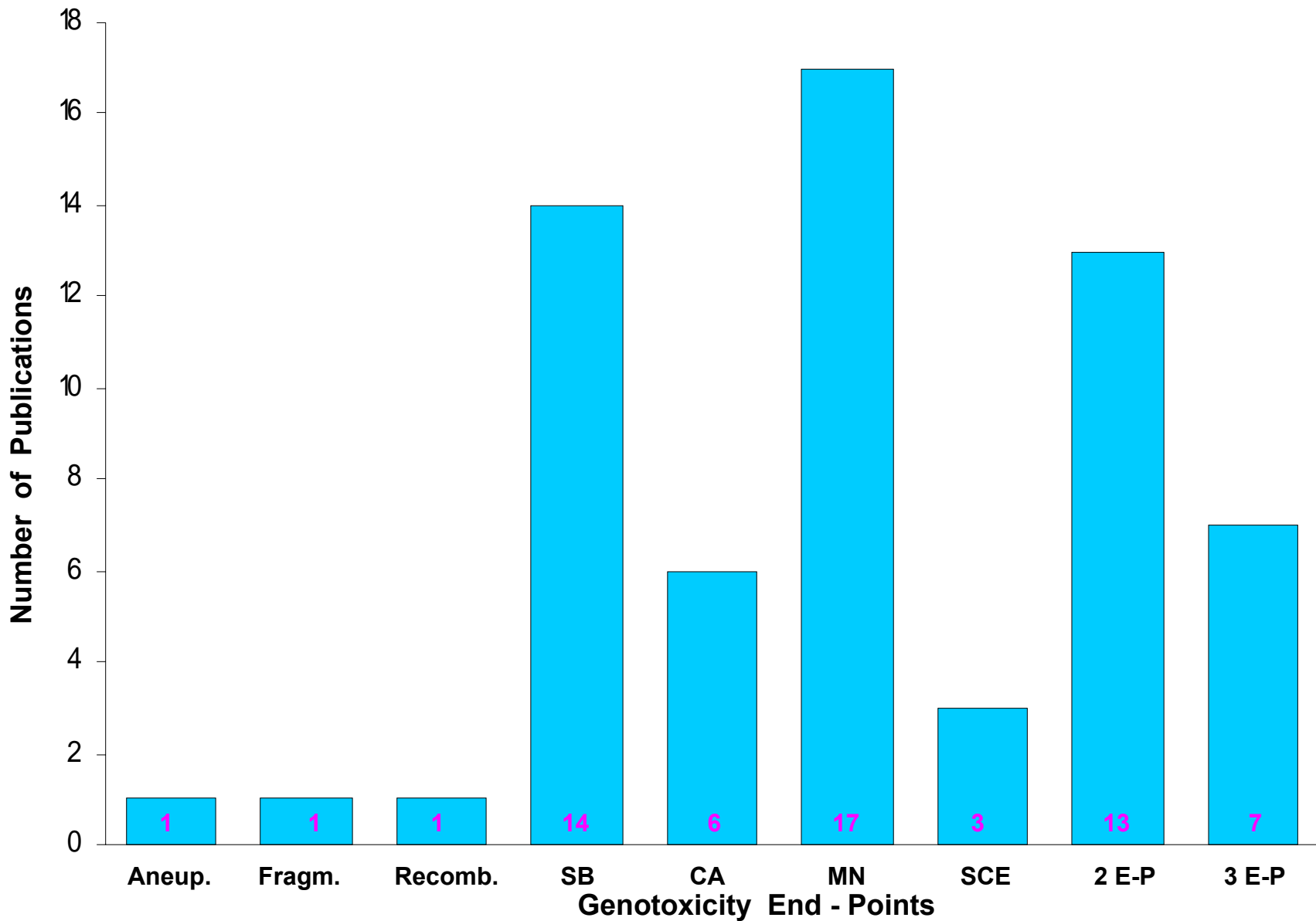
1990 - 2005 Publications

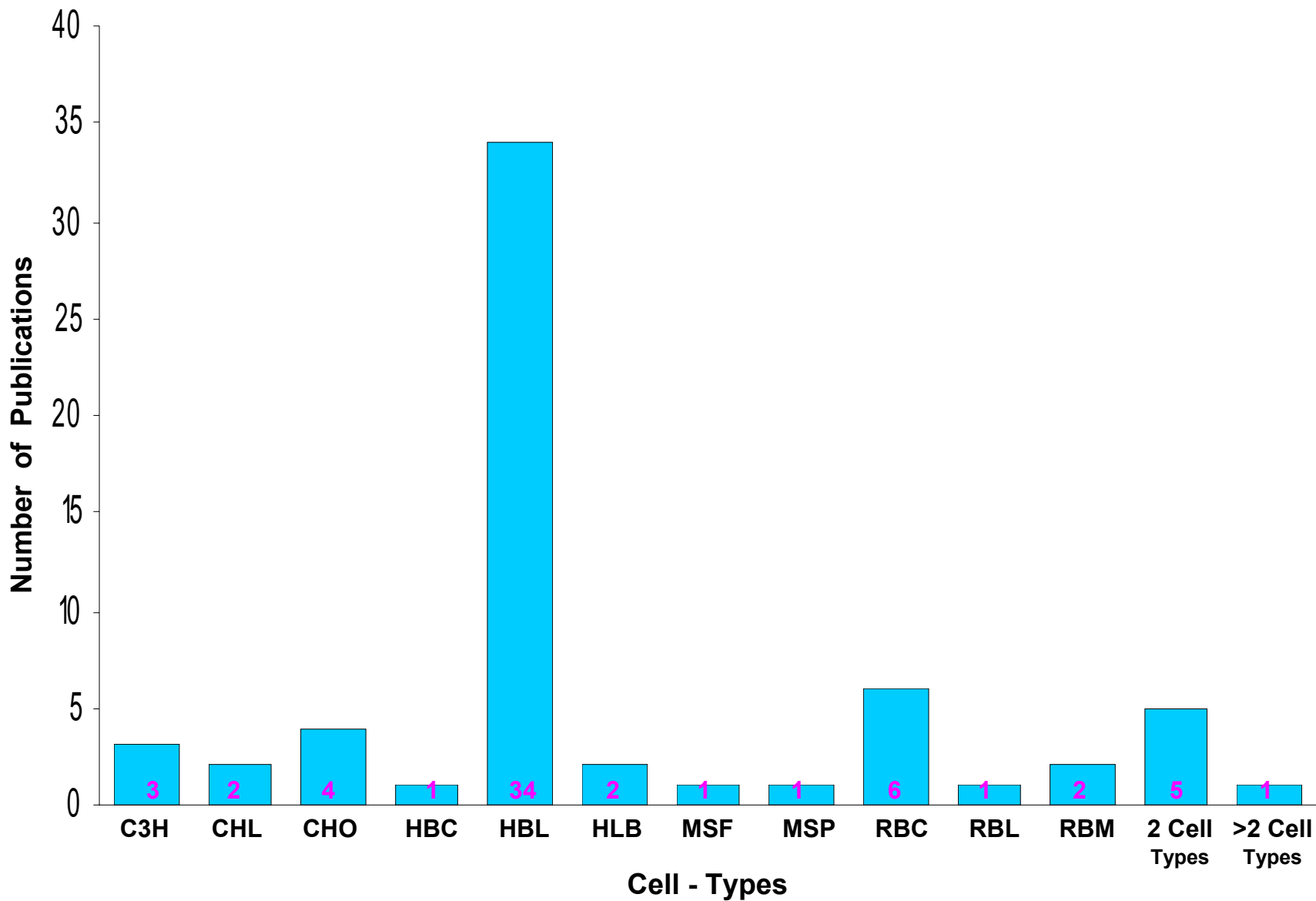
Sr#	First Author	Sr#	First Author	Sr#	First Author
1	Garaj-Vrhovac, 1990a	22	Vijayalaxmi, 1997a	43	Trosic, 2002
2	Garaj-Vrhovac, 1990b	23	Vijayalaxmi, 1997b	44	Gadhia, 2003
3	Kerbacher, 1990	24	Malyapa, 1998	45	Koyama, 2003
4	Ciaravino, 1991	25	Phillips, 1998	46	McNamee, 2003
5	Garaj-Vrhovac, 1991	26	Garaj-Vrhovac, 1999	47	Mashevich, 2003
6	Garson, 1991	27	Maes, 2000	48	Vijayalaxmi, 2003a
7	Fucic, 1992	28	Vijayalaxmi, 2000	49	Vijayalaxmi, 2003b
8	Garaj-Vrhovac, 1992	29	Zotti-Martelli, 2000	50	Zeni, 2003
9	Garaj-Vrhovac, 1993	30	Lalic, 2001	51	Hook, 2004
10	Maes, 1993	31	Li, 2001	52	Koyama, 2004
11	Sarkar, 1994	32	Maes, 2001	53	Lagroye, 2004a
12	d'Ambrosio, 1995	33	Sykes, 2001	54	Lagroye, 2004b
13	Lai, 1995	34	Vijayalaxmi, 2001a	55	Trosic, 2004
14	Maes, 1995	35	Vijayalaxmi, 2001b	56	Baohong, 2005
15	Lai, 1996	36	Vijayalaxmi, 2001c	57	Diem, 2005
16	Maes, 1996	37	d'Ambrosio, 2002	58	Gandhi, 2005a
17	Antonopoulos, 1997	38	Bisht, 2002	59	Gandhi, 2005b
18	Lai, 1997	39	McNamee, 2002a	60	Gorlitz, 2005
19	Maes, 1997	40	McNamee, 2002b	61	Komatsubara, 2005
20	Malyapa, 1997a	41	Mei-Bian, 2002	62	Zeni, 2005
21	Malyapa, 1997b	42	Tice, 2002	63	Zotti-Martelli, 2005

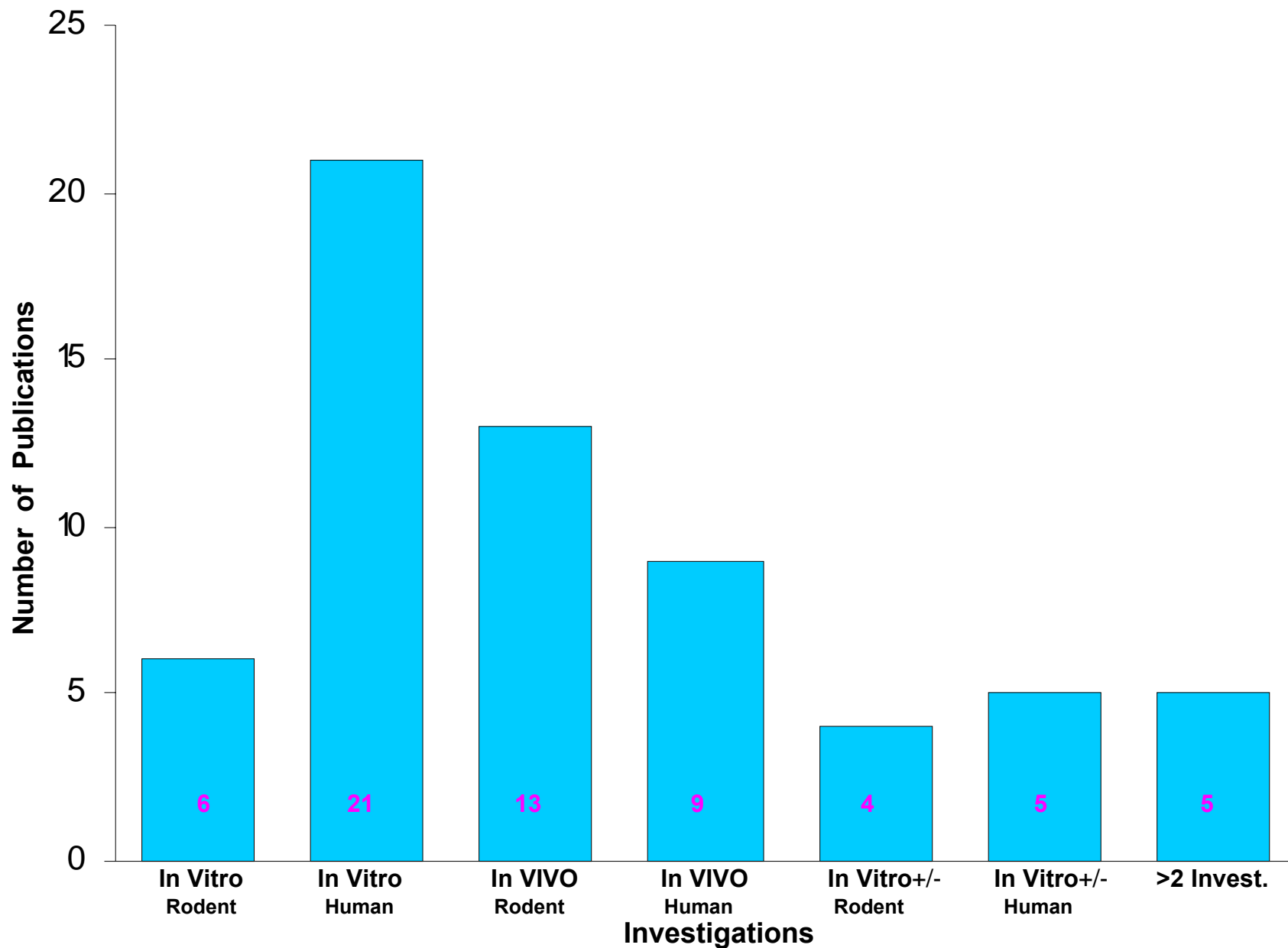












Meta-Analysis Data Base

All published data were considered

(Final xls. spread sheet contained 922 rows & 60 columns)

Three specific RFR exposure characteristics

Frequency (MHz) ≤ 2000 & > 2000

SAR (W / kg) ≤ 2 , $> 2-5$ & > 5

CW / PW / CP

Standardized Units

DNA strand breaks - comet tail length	Microns
Chromosomal Aberrations	/ 100 cells
Micronuclei	/ 1000 cells
Sister Chromatid Exchanges	/ 1 cell

Pooled Data

For each genotoxicity end-point, each experiment was 'weighted' equally
(sample size taken consideration)

Data from sham / un-exposed controls were pooled as cells in Control Group (C)

Data from RFR-exposed cells were pooled as cells in Experimental Group (E)

to obtain the consolidated mean, SD & 95% confidence interval

Meta-Analysis

E – C

**Magnitude of Difference between Cells
in Experimental and Control Groups**

(variability & sample size taken into consideration)

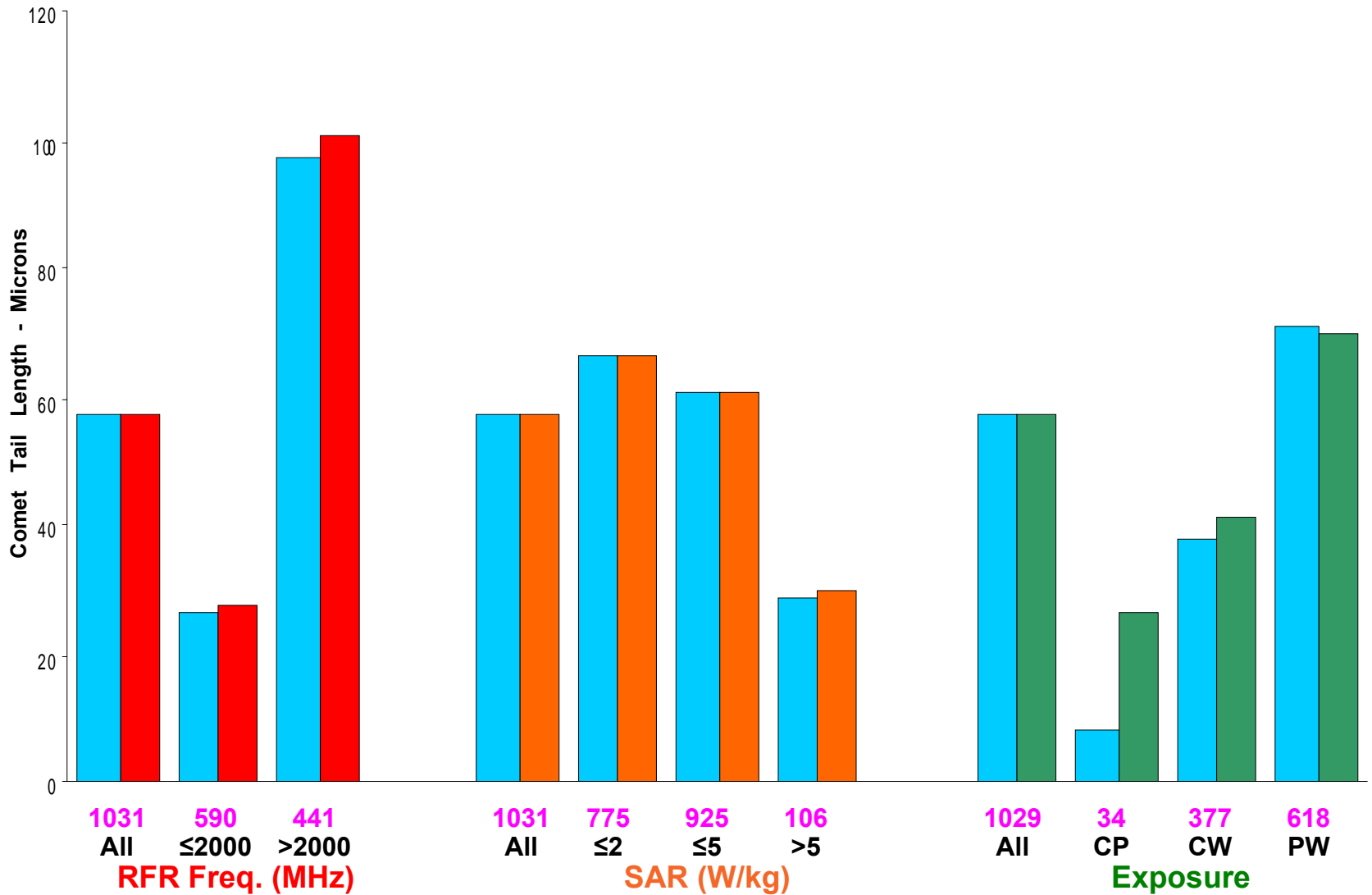
ES (Effect Size)

$$\frac{\text{Experimental Group Mean} - \text{Control Group Mean}}{\text{Pooled SD of Control and Experimental Groups}}$$

(variability, sample size & units taken into consideration)

DNA Strand Breaks

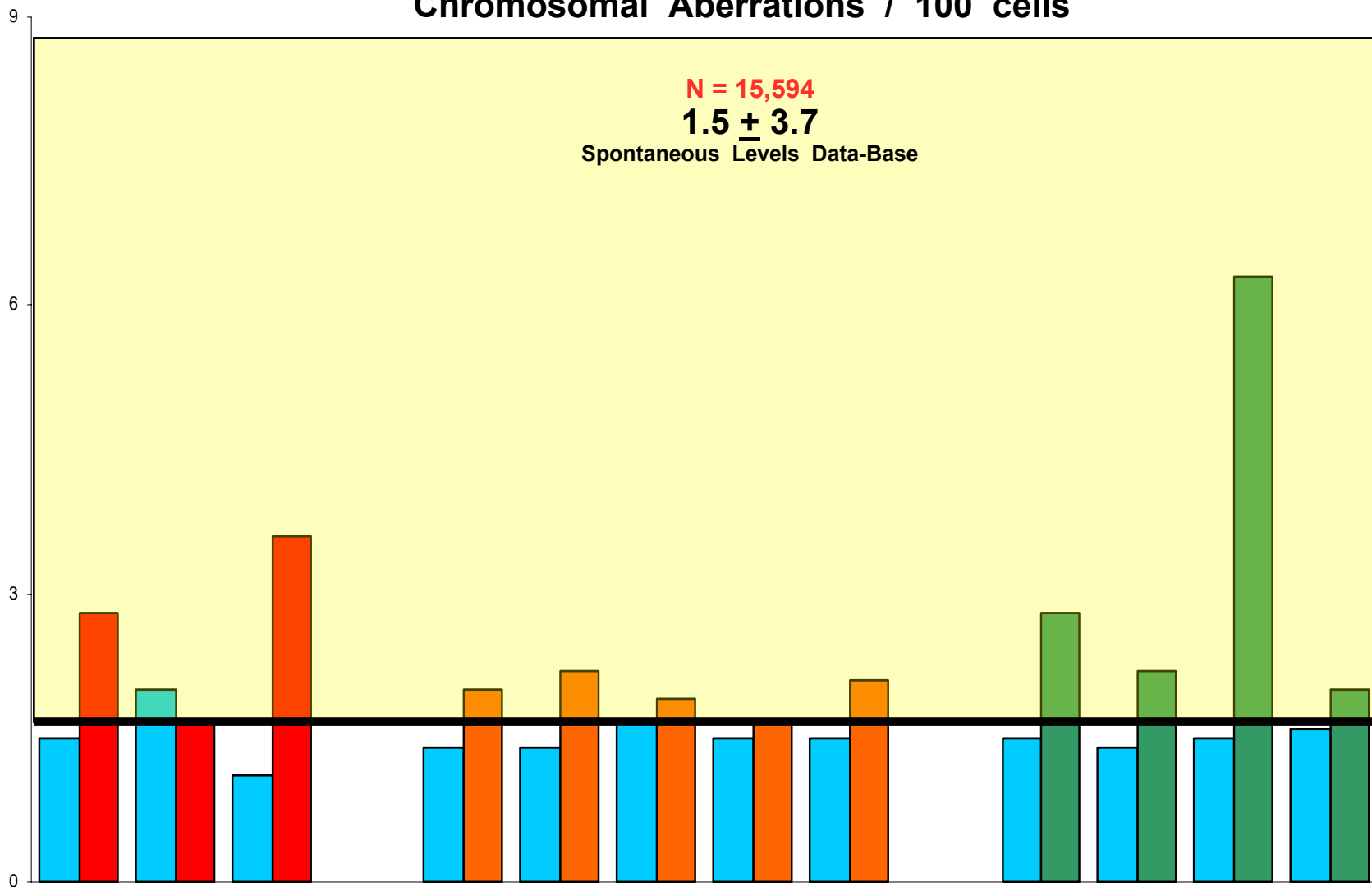
Comet Tail Length - Microns



Chromosomal Aberrations / 100 cells

N = 15,594
1.5 ± 3.7
 Spontaneous Levels Data-Base

Chromosomal Aberrations / 100 cells

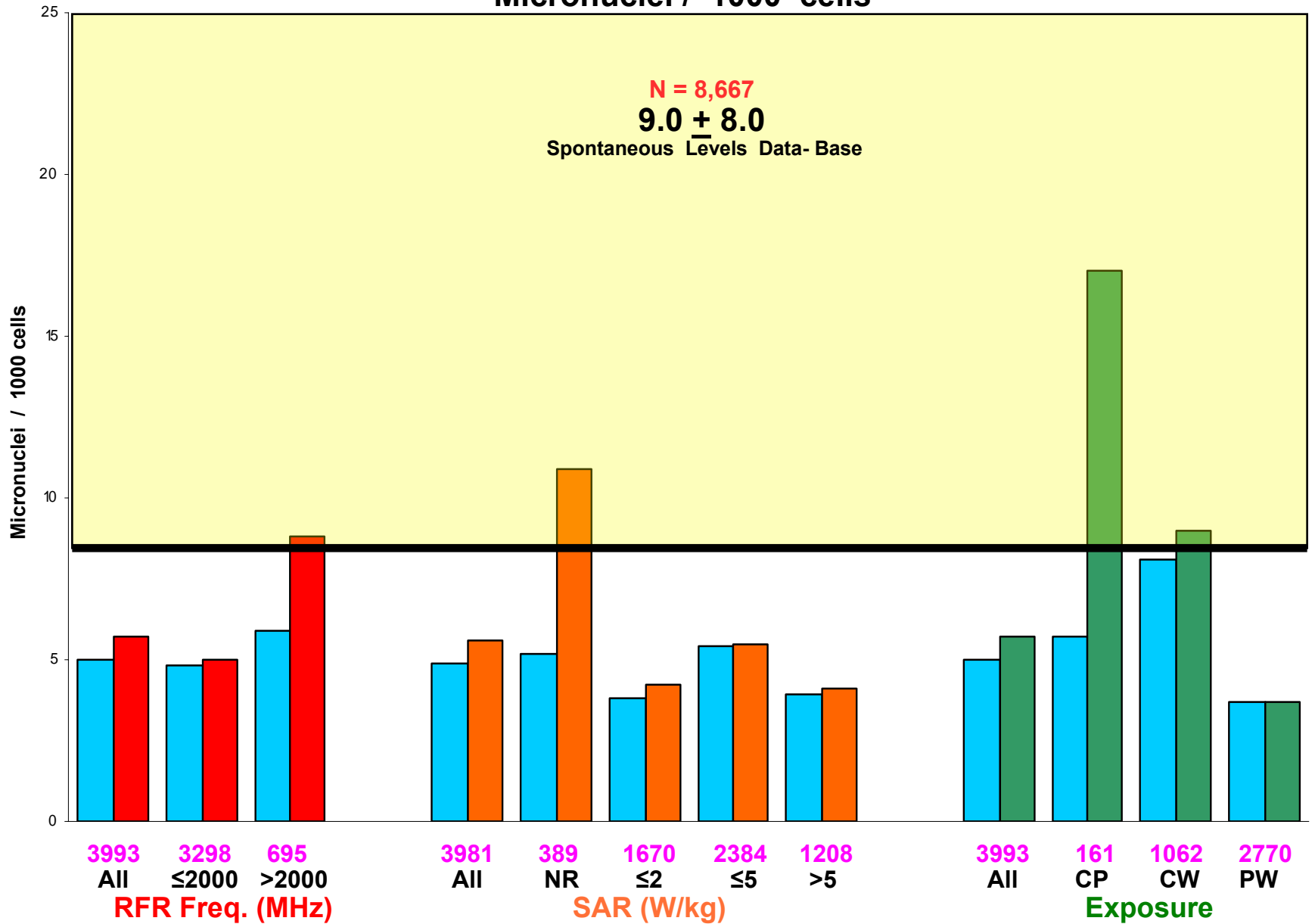


Micronuclei / 1000 cells

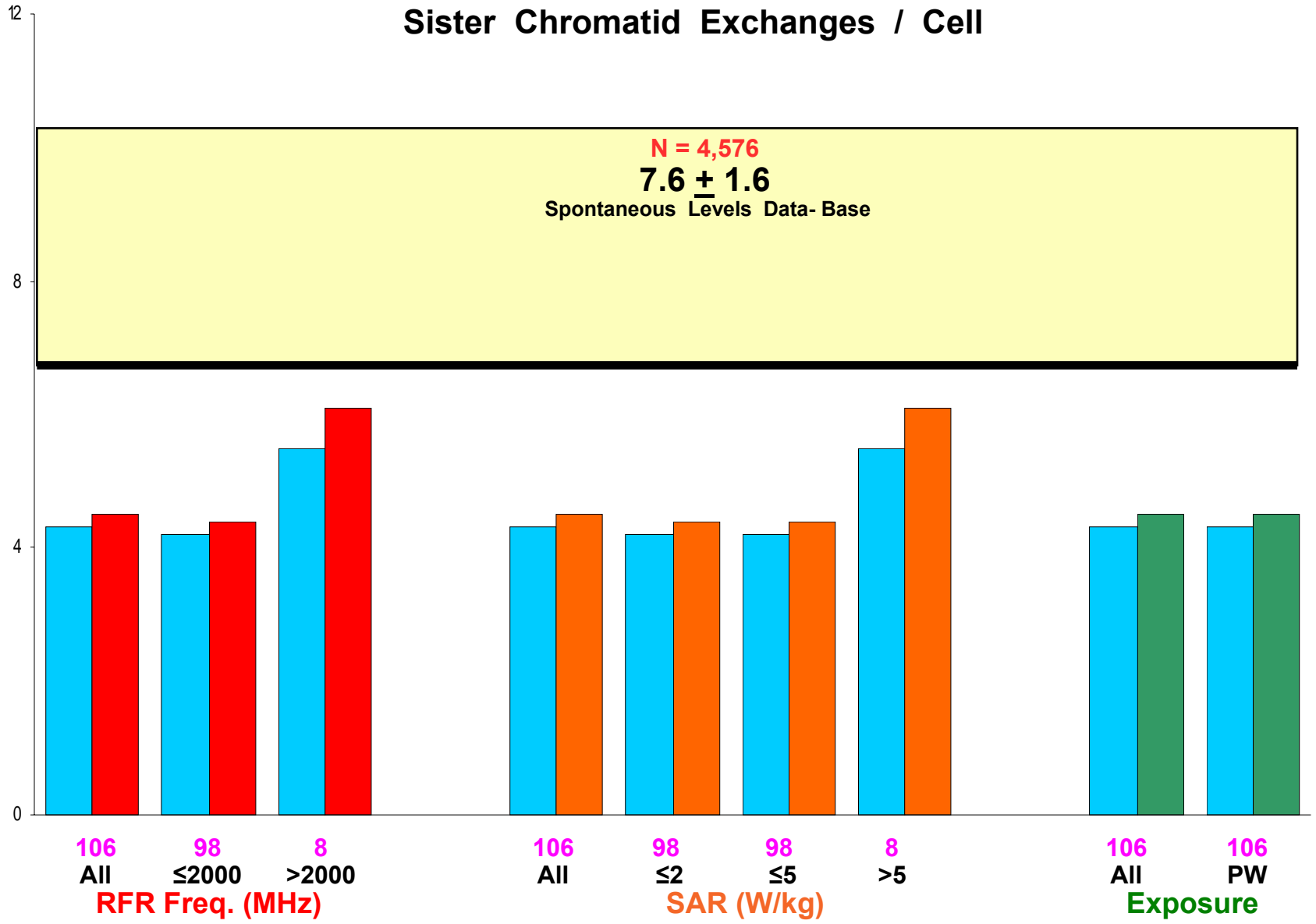
N = 8,667

9.0 + 8.0

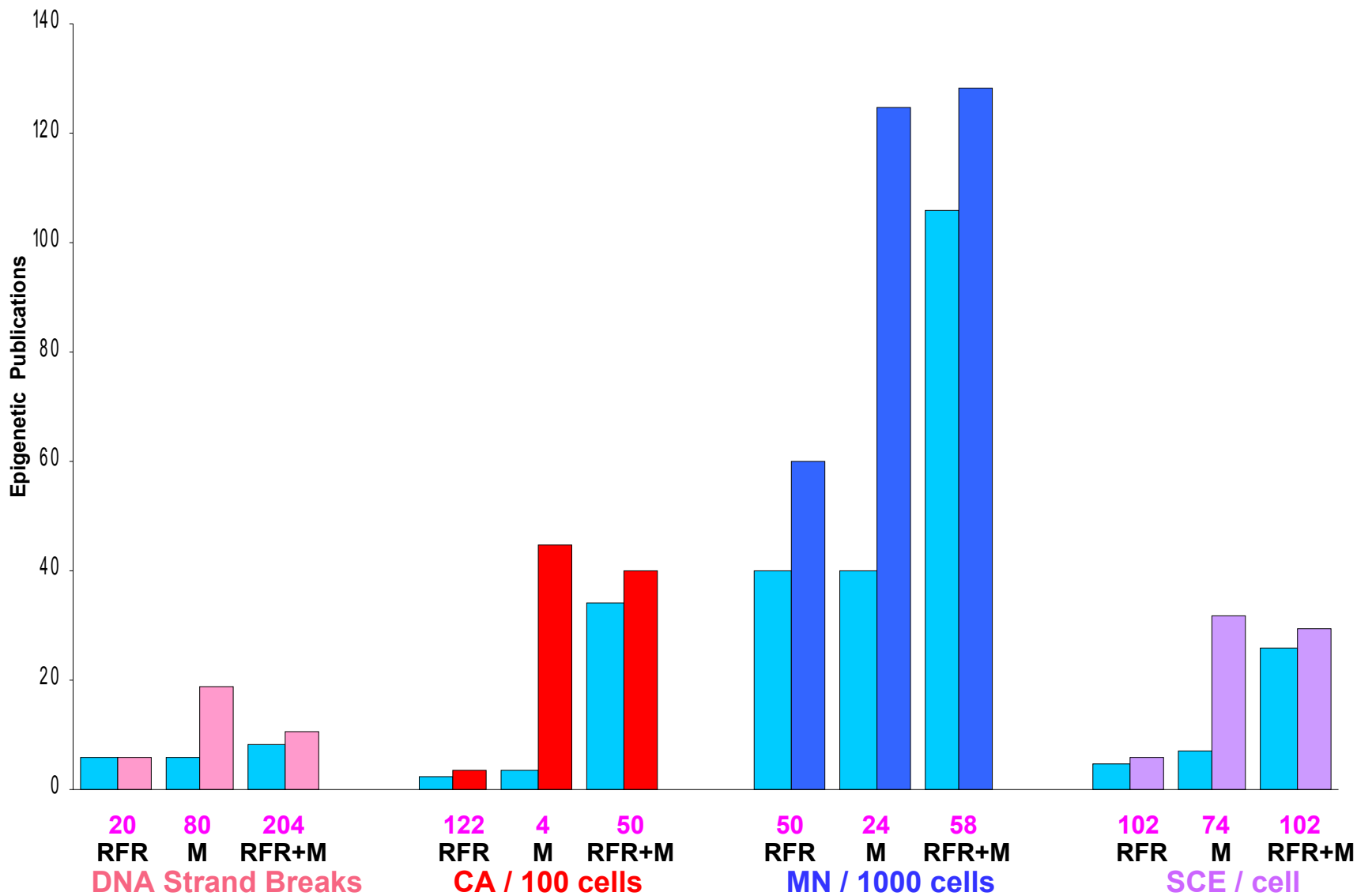
Spontaneous Levels Data-Base



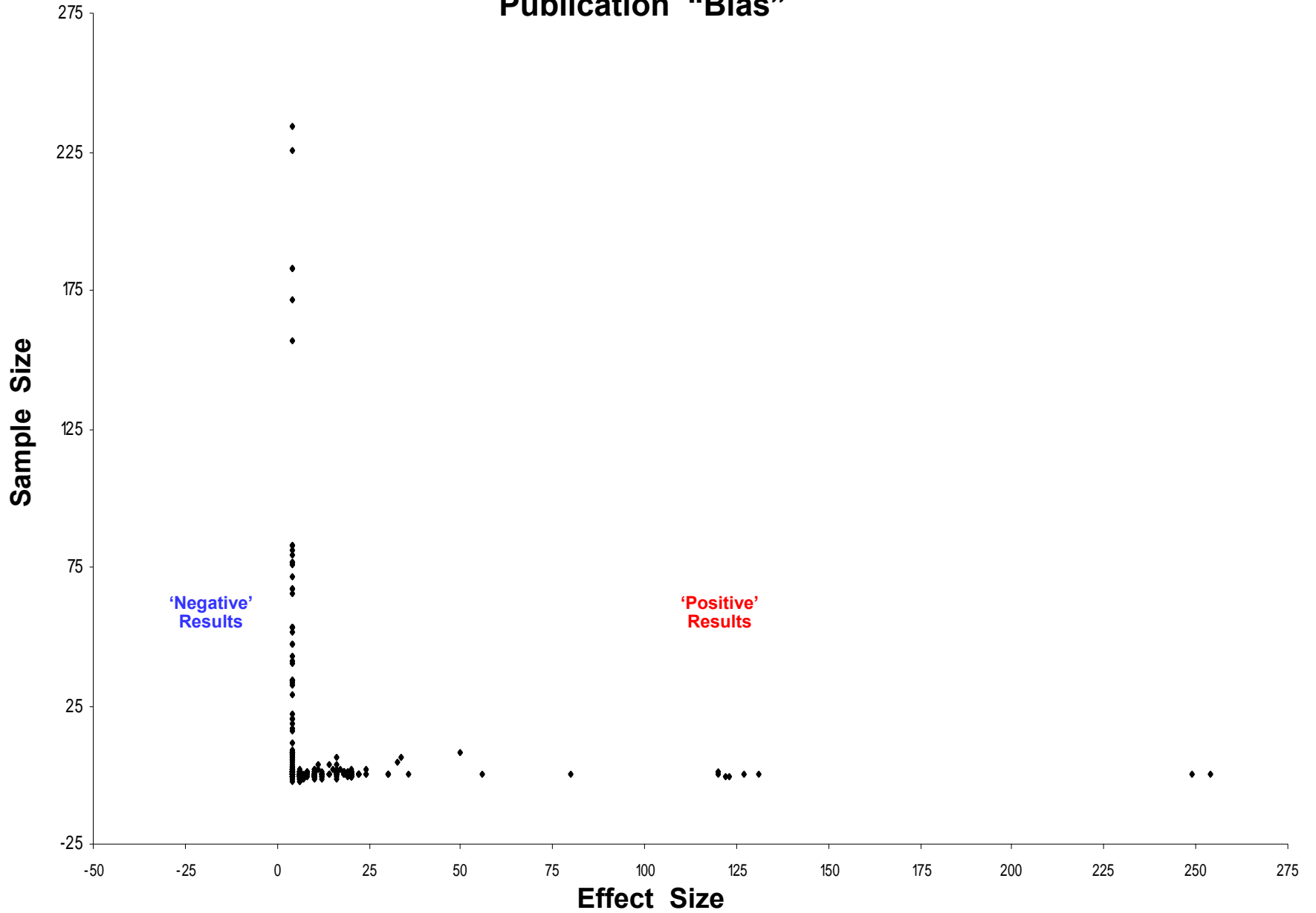
Sister Chromatid Exchanges / Cell



Epigenetic Investigations



Publication "Bias"



Conclusions - Meta-Analysis

Difference between RFR-exposed and sham-/unexposed cells as well as the 'effect size' due to RFR exposure was small

At certain RFR exposure conditions there was a statistically significant increase in some genotoxicity end-points

The mean indices for CA, MN and SCE in RFR-exposed and sham-/unexposed cells were within the spontaneous levels reported in historical data-base

Considerable evidence for publication bias

Genotoxicity End-points

Biomarkers

Adverse Human Health Risk Assessment

?

DNA Strand Breaks

No Systematic Analysis

Difficulties in integrating the data from

Various Researchers

Different Protocols

Different Evaluations Procedures

DNA Repair was NOT assessed

No Sig. Increase in RFR-exposed Cells

Chromosomal Aberrations

Most Reliable Biomarker

Aberration Frequencies Increase Prior to
Clinical Manifestation of the Disease

No Sig. Increase in RFR-exposed Cells

Micronuclei

Preliminary Evidence

More than one mechanism

No Sig. Increase in RFR-exposed Cells

Sister Chromatid Exchanges

No Predictive Value

Mechanism(s) & Significance ?
Induction by common chemicals
Influence of cell culture conditions

No Sig. Increase in RFR-exposed Cells

SCE shall remain as valuable Genotoxicity end-point
among the short-term assays

ICNIRP & IEEE Safety Level

(based on 4 W / kg SAR – threshold for heat generation)

Occupational	0.4 W / kg SAR (1/10 th safety factor)
General Public	0.08 W / kg SAR (1/50 th safety factor)
Localized Expo.	1.6 W / kg SAR (brain in mobile phone users)

at these Recommended Safety Levels

DATA from Meta-Analysis
No Sig. Increase in Genotoxicity in
RFR-exposed Cells

Evaluated by Various End-points
(including the most reliable Biomarker - Chromosomal Aberrations)

Vijayalaxmi & Prihoda., Radiation Research. 169, 561 – 574, 2008

FUTURE
?