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THE
NON-CLINICAL
ENGINE
SFT 2019

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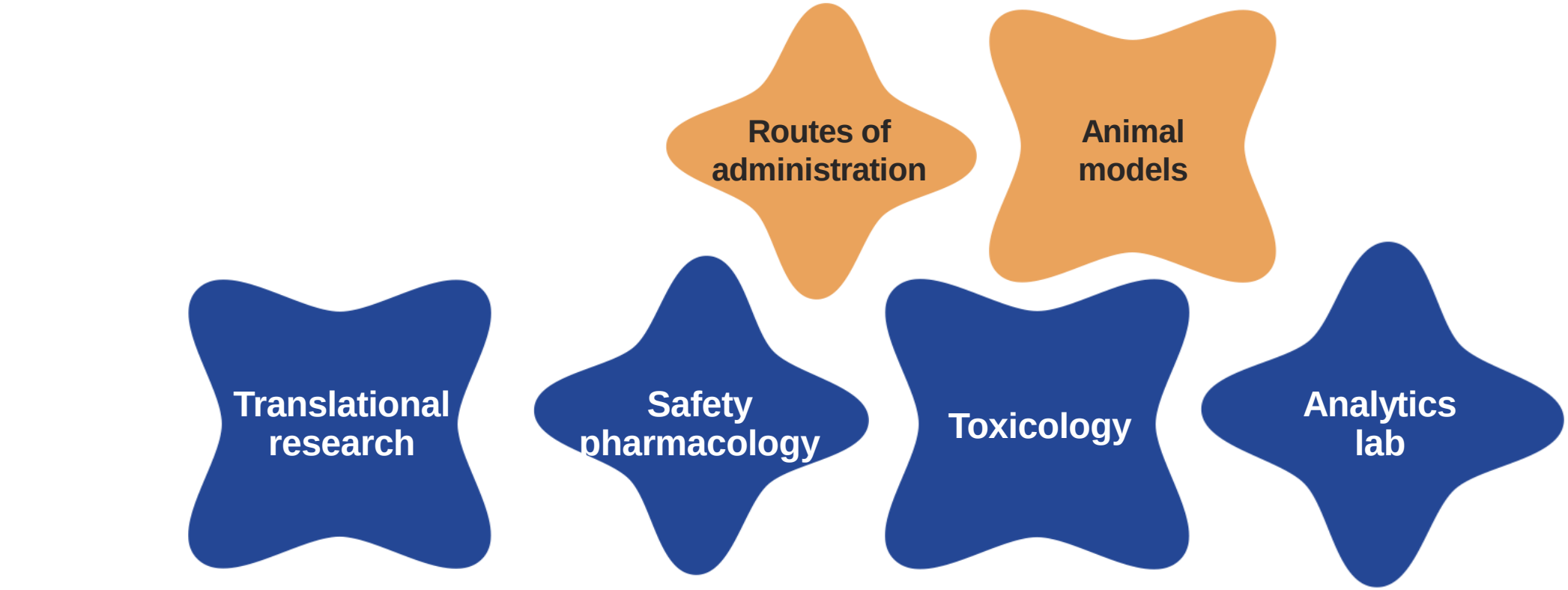
ERBC Overview

European leader in non-clinical studies

- Joined offer of 2 reference CROs in safety pharmacology and toxicology studies: CERB (Baugy, France) and RTC (Roma, Italy)
- Full engine covering the non-clinical studies of any therapies or chemical compound, from preclinical proof-of-concept to market
- Comprehensive suite of experimental capabilities, preclinical models, regulatory pre-IND package and consultancy services
- Multidisciplinary team of experts (200 FTE), including leaders in safety cardiology, DART, electrophysiology and pathophysiology
- World class academic and industrial network
- 15,000 m2 facilities, with more than half dedicated to laboratories and animal housing
- Deep commitment in ethics and animal welfare

Thousands of study reports successfully used in support of market authorization around the world

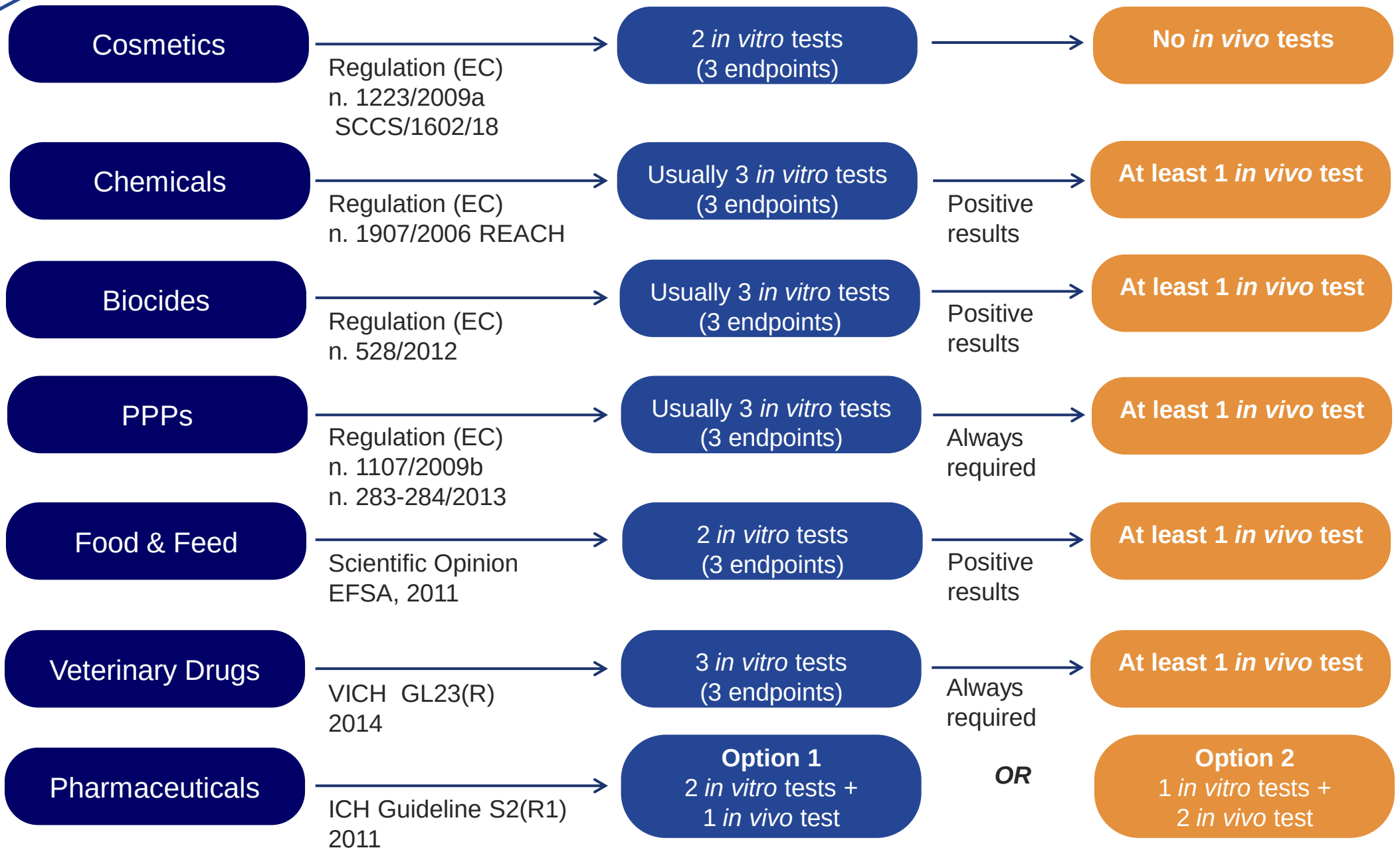
The non-clinical engine



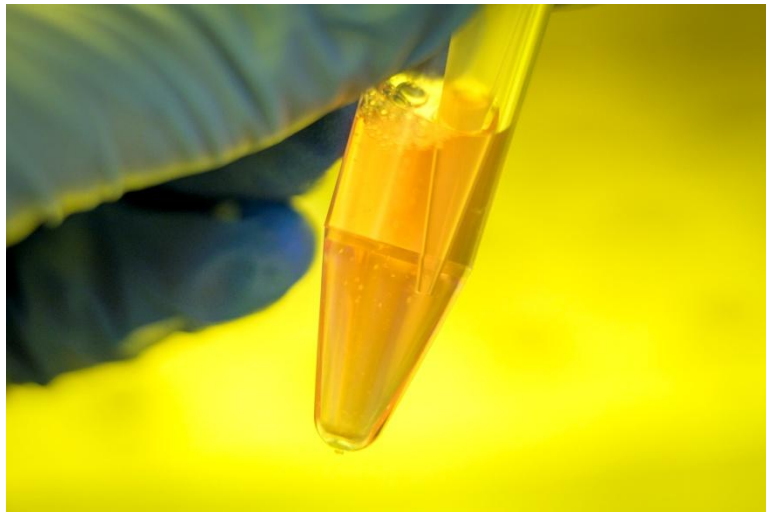
THE NON-CLINICAL ENGINE

Performance improvement for *in vitro* genotoxicity testing: the experience of a Contract Research Organization

Serena Cinelli


Cosmetics
Regulation (EC) n. 1223/2009a SCCS/1602/18
2 in vitro tests (3 endpoints)
No in vivo tests
Chemicals
Regulation (EC) n. 1907/2006 REACH
Usually 3 in vitro tests (3 endpoints)
Positive results
At least 1 in vivo test
Biocides
Regulation (EC) n. 528/2012
Usually 3 in vitro tests (3 endpoints)
Positive results
At least 1 in vivo test
PPPs
Regulation (EC) n. 1107/2009b n. 283-284/2013
Usually 3 in vitro tests (3 endpoints)
Always required
At least 1 in vivo test
Food & Feed
Scientific Opinion EFSA, 2011
2 in vitro tests (3 endpoints)
Positive results
At least 1 in vivo test
Veterinary Drugs
VICH GL23(R) 2014
3 in vitro tests (3 endpoints)
Always required
At least 1 in vivo test
Pharmaceuticals
ICH Guideline S2(R1) 2011
Option 1 2 in vitro tests + 1 in vivo test
OR
Option 2 1 in vitro tests + 2 in vivo test

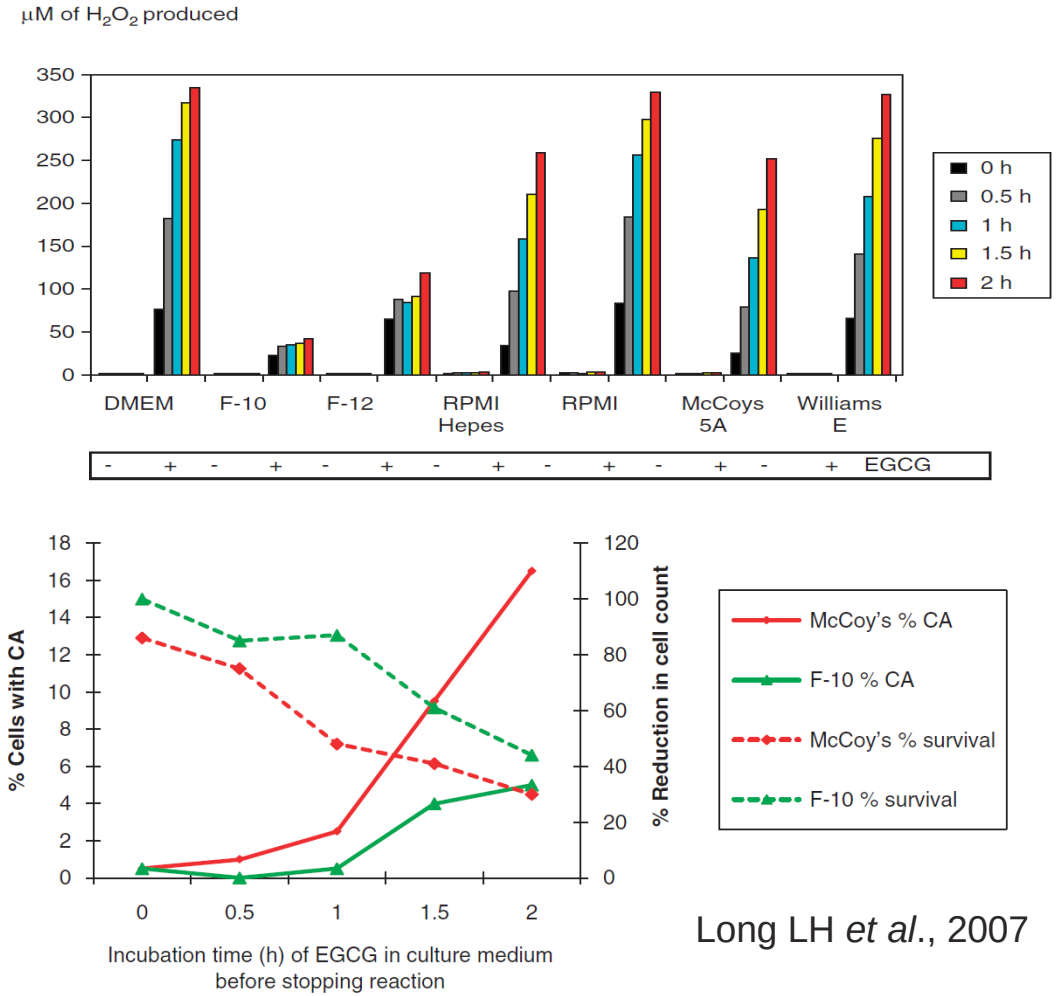
Importance of reducing false positive results



- Artifacts of *in vitro* cell culture conditions
- Stability of cell lines
- Impaired p53 function and altered DNA repair capability in the rodent cell lines commonly used
- Adequate levels and measurements of cytotoxicity

Artifacts of *in vitro* cell culture conditions

Polyphenols such as epigallocatechin gallate and ascorbic acid have been shown to interact with components in cell culture medium leading to production of hydrogen peroxide



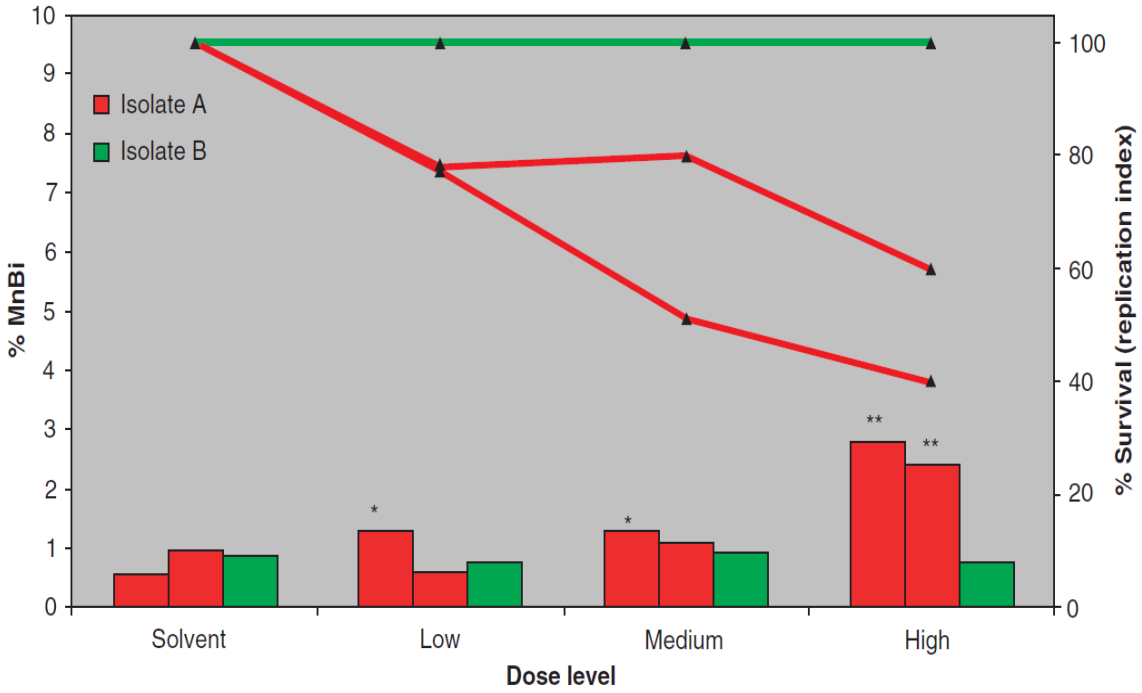
Long LH *et al.*, 2007

Rapid production of hydrogen peroxide by interaction of 1 mM epigallocatechin gallate with different culture media

Induction of cytotoxicity and chromosome aberrations in cultured CHO using two different culture media

Stability of cell lines

MN responses in two different isolates of L5178Y cells treated with o-anthranilic acid



Isolate A (obtained from a commercial source): cells were examined and were found to have an abnormal karyotype and a very high frequency of translocations

Isolate B (obtained from a private source): known to be of an earlier passage closer to the original derivation, it was neither toxic nor induced MN even at 10 mM

P. Fowler *et al.*/Mutation Research 742 (2012)11-25

Stability of cell lines

Overall, the source of the cell line, as the history/handling of the cells may influence their characteristics and, consequently, the results of the assays

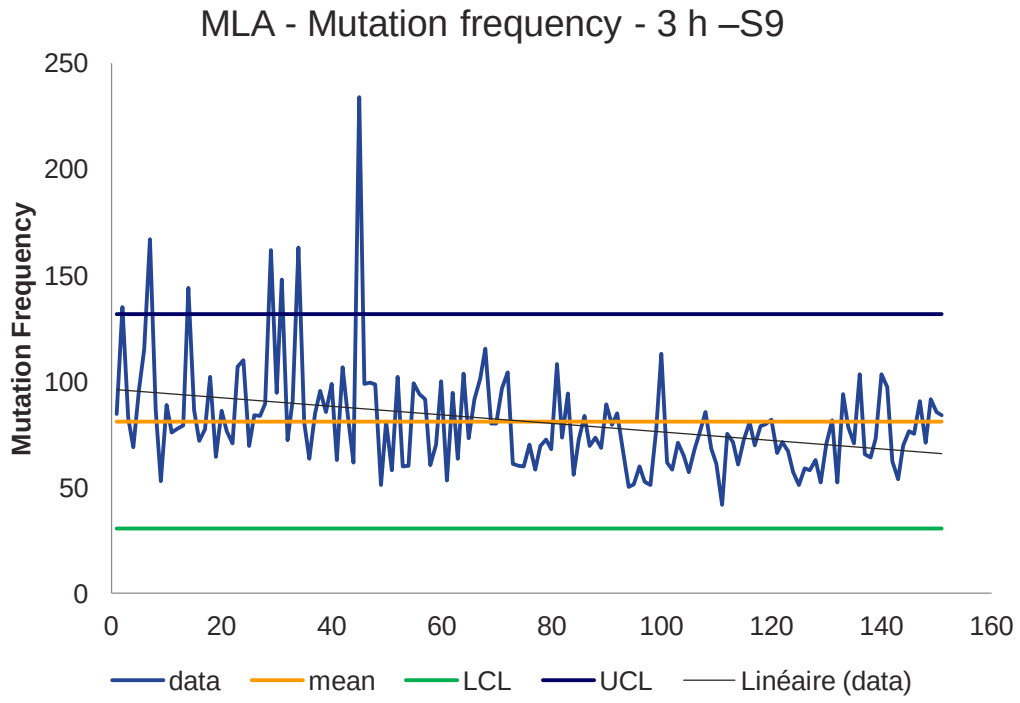
Good Cell Culture Practices (GCCP) are of critical importance for the relevance of results obtained using in vitro cell systems

Historical control data

Contents lists available at ScienceDirect
Mutation Research/Genetic Toxicology and Environmental Mutagenesis
journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Compilation and use of genetic toxicity historical control data

M. Hayashi *et al.* 2011, MR



Historical control data should be used:

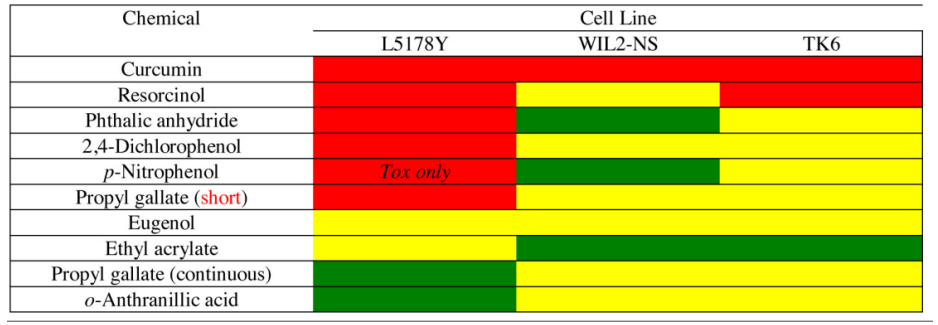
- for interpretation of the results obtained in each experiment (biological relevance)
- to define acceptance limits
- **to keep the cell line under control**

Cell line properties

Chemical ("misleading" positives from Kirkland et al 2008)	Outcome of in vitro Mn test Values indicate lowest positive dose for MN induction (µg/mL)											
	p53 compromised cell types						p53 functional cell types					
	V79		CHO		CHL		HuLy		TK6		HepG2	
Pthallic anhydride	Neg	Neg	Neg	Neg	1480	1380	Neg	Neg	575	725	1481	Neg
2,4 Dichlorophenol	8.5	8.6	27	23	145	155	Neg	187	Neg	Neg	Neg	Neg
Curcumin	4	6	12	17	14	19	31	35	16	16	Neg	Neg
Ethyl acrylate 3+21hours	16	20	32	20	39	40	38	50	25	20	Neg	96
Ethyl acrylate 24+0 hours	1	4	Neg	Neg	7	14	Neg	Neg	Neg	10	Neg	35
1,3 Dihydroxybenzene (resorcinol)	196	261	480	444	307	319	Neg	Neg	Neg	Neg	Neg	377
p-nitrophenol	Neg	Neg	569	950	1043	500	Neg	Neg	Neg	Neg	Neg	782
Eugenol	13	9	19	50	220	180	Neg	Neg	Neg	160	Neg	Neg
Propyl gallate	5	8	11	10	24	48	Neg	Neg	4	21	Neg	Neg
Sodium xylene sulfonate	1562	1666	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	1481
Tertiary-butylhydroquinone	21	20	60	89	55	25	Neg	Neg	19	42	310	Neg
D,L-menthol	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
0-Anthranilic acid	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Ethionamide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Urea	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

The rodent cell lines (V79, CHO and CHL) were consistently more susceptible to cytotoxicity and MN induction than human p53-competent cells

P. Fowler *et al*./Mutation Research 742 (2012)11-25



Differences between human and rodent cell lines were not primarily due to the p53 status but more likely to some elements of species difference (DNA repair capabilities, cell cycle control etc.)

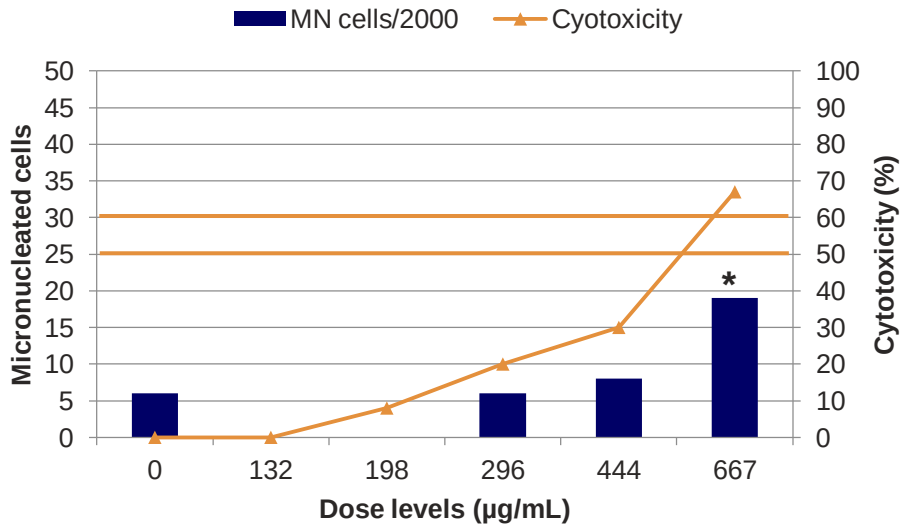
J. Withwell *et al*./Mutation Research 789-790 (2015) 7-27

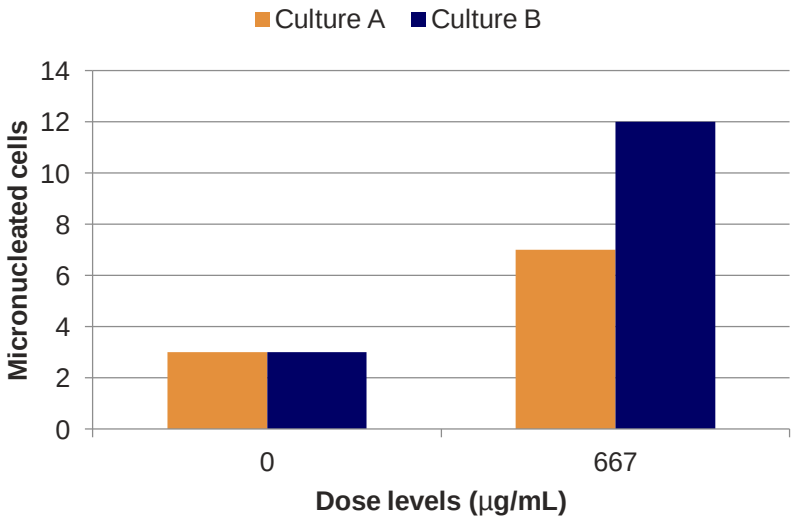
Extent of cytotoxicity

Excessive toxicity can result in:

- **“false” negative results** if cell growth is delayed so that they do not reach metaphase, or they fail to progress through metaphase into the next interphase where micronuclei can be detected
- **“misleading” positive results** that occur only under cytotoxic conditions and not at lower concentrations. The events that lead to chromosome aberrations originate directly or indirectly from the processes that result in toxicity

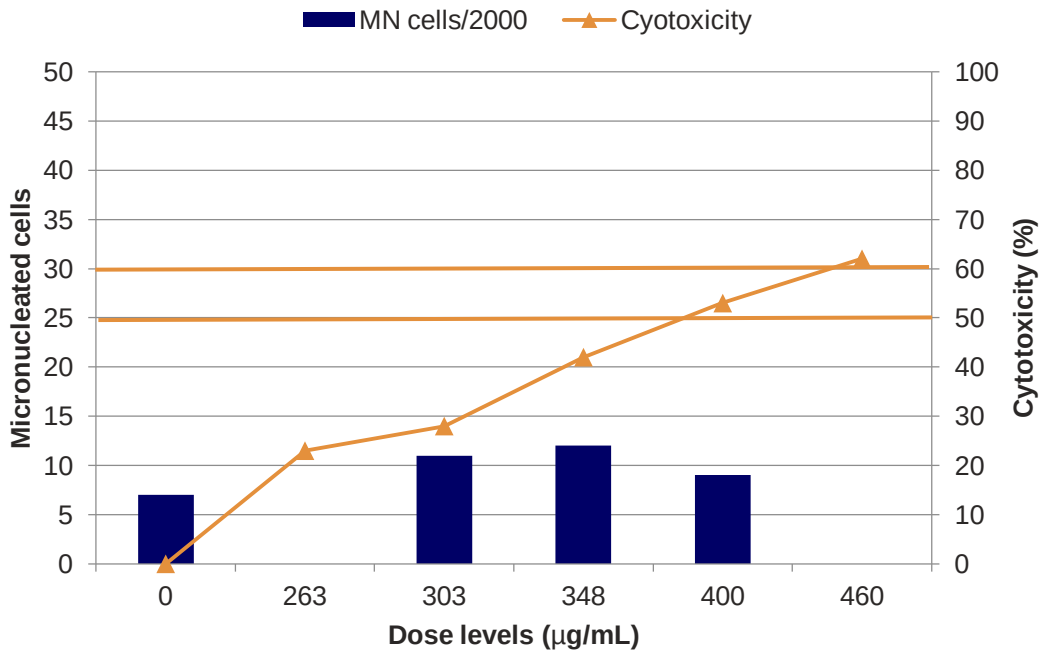
Case Study 1





Extent of cytotoxicity

Case Study 1

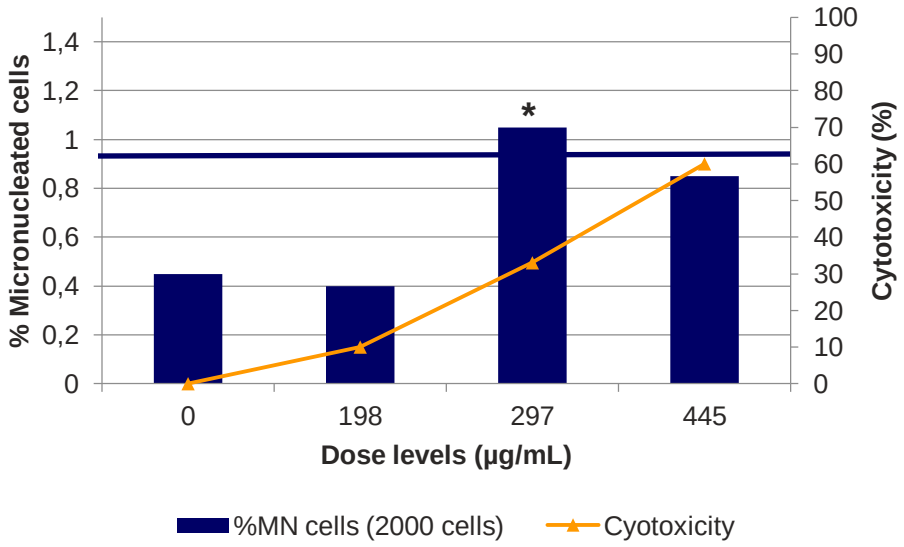


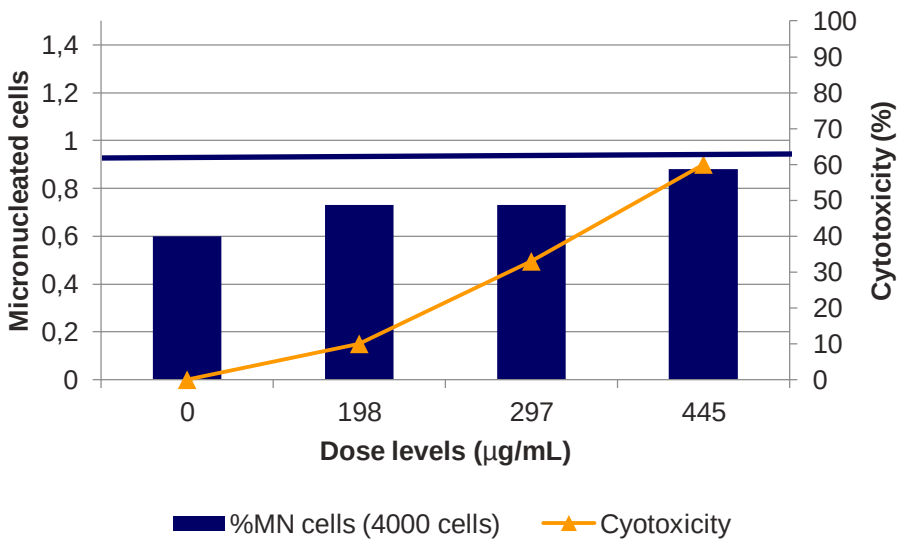
Repeated experiment:

- adequate toxicity
- no increase in the incidence of micronucleated cells
- no result fell out the historical control range
- no linear trend was indicated

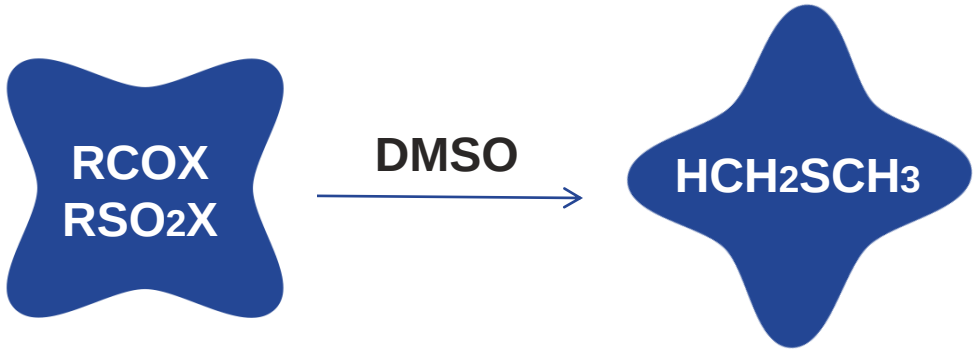
Sample size

Case Study 2 *in vitro* micronucleus test





Choice of solvent



Solvents should be chosen carefully, taking into account their potential to react with test chemicals.

DMSO has been shown to react with some classes of chemicals such as carboxilic/sulfonic acid halides.

These were positive in bacterial reversion tests due to reaction with DMSO to form dimethylsulfide halides.

These are alkilating agents and sufficiently stable to produce positive results; when tested in water the results were negative

A. Amberg *et al.*/Org. Process Res.Dev. 19 (2015) 1495-1506

Ames positive results

Factors affecting chemical accessibility, metabolism and toxicity in bacteria, mammalian cells and intact mammals.

Bacteria	Mammalian cells	Mammals
Circular DNA (no nucleus)	Nuclear chromosomes with associated proteins and histones	Nuclear chromosomes with associated proteins and histones
Cell wall	Plasma membrane	Plasma membrane
Single cell, exposure *via* immediate environment	Single cell or monolayers, exposure *via* solution	Tissue and organs, exposure *via* ADME (bloodstream)
Limited xenobiotic metabolism	Limited xenobiotic metabolism	Extensive xenobiotic metabolism
Limited antioxidant activity	Some antioxidant activity	Full antioxidant activity
Response to stress/toxicity is mutation	Response to stress/toxicity is cell death	Response to stress/toxicity is cell death
Default to a toxic environment is survival through mutation	Default to a toxic environment is cell death	Default to a toxic exposure is cell death
Response to toxicity is dose dependent	Response to toxicity is dose-dependent with a low threshold	Response to toxicity is dose-dependent with highest possible threshold
DNA damage repair is geared to survival/mutation	DNA damage repair is geared to fidelity	DNA damage repair is geared to fidelity

D. Kirkland et al., 2014

Bacteria and mammalian cells have different capabilities to cope with specific oxidative stress

- **addition of antioxidants**
- **specific biochemical assays addressing oxidative stress**

Ames positive results

The intrinsic metabolism of the bacterial and mammalian cells may produce different metabolic profiles

S9 mix → oxidative metabolism
bacteria may be less able to defend against oxidative metabolites, whereas mammalian cells may have sufficient phase II metabolism to promote detoxification

Addition of cofactors for phase II metabolism

Metabolic profiling using induced rat hepatocytes and human hepatocytes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Thank you