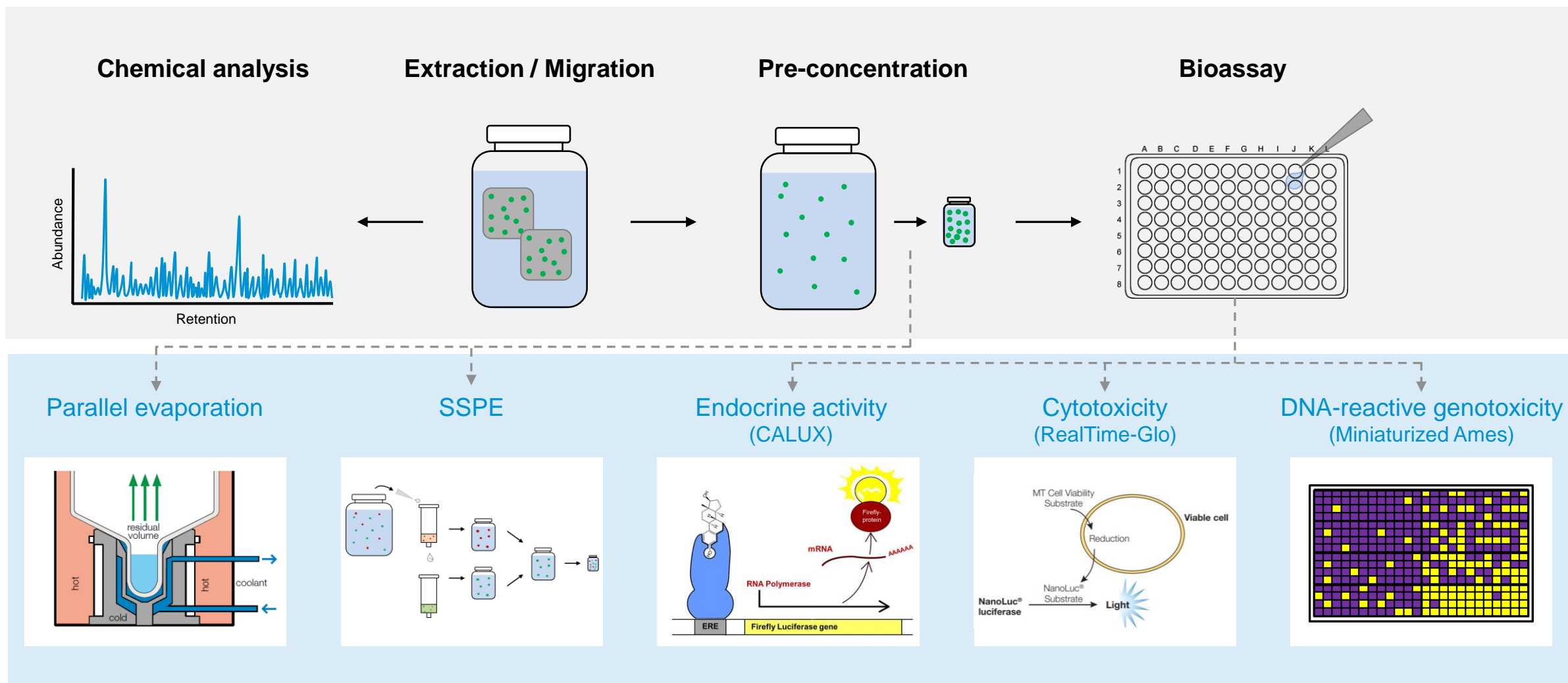




## Introduction of the final Migratox method



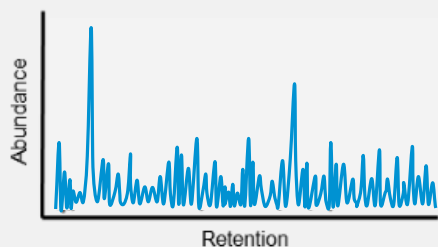
# Workflow: "Migratox method"





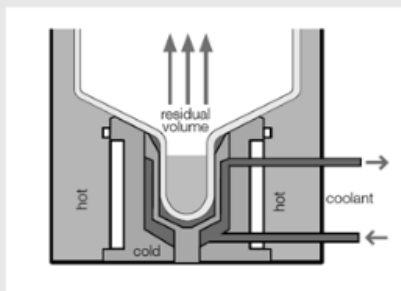
# Workflow: “Migratox method”

## Chemical analysis

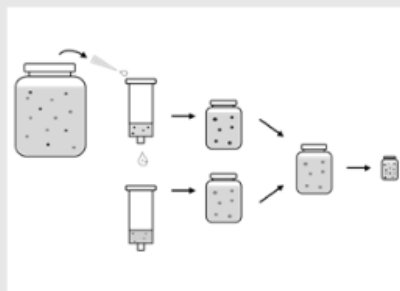


- Methods for **chemical analysis** & to detect **endocrine activity** already developed in previous projects (Xeno, Senses)
- Methods were included in Migratox method, but were not the primary focus of research

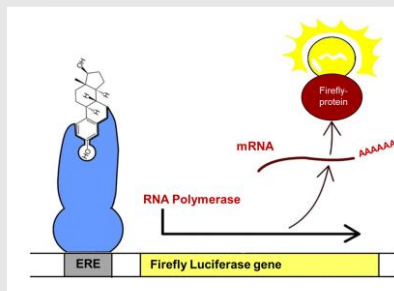
## Parallel evaporation



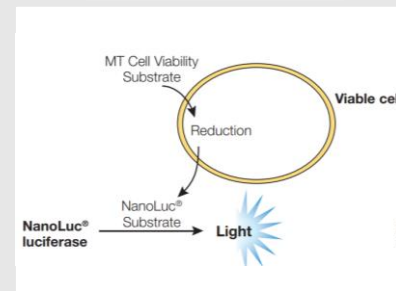
## SSPE



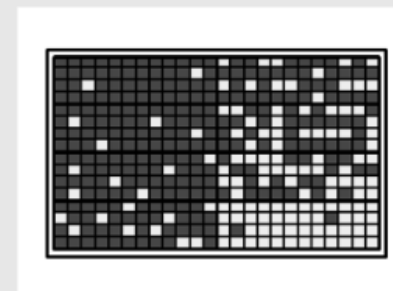
## Endocrine activity (CALUX)



## Cytotoxicity (RealTime-Glo)



## DNA-reactive genotoxicity (Miniaturized Ames)





# Workflow: “Migratox method”

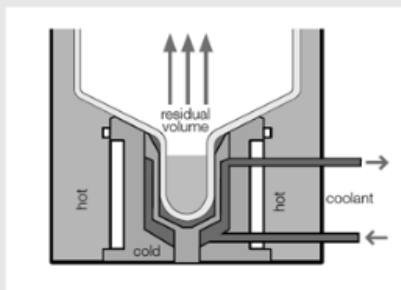
Evaluation of:

- 8 different genotoxicity tests
- > 60 model substances for genotoxicity test development and validation

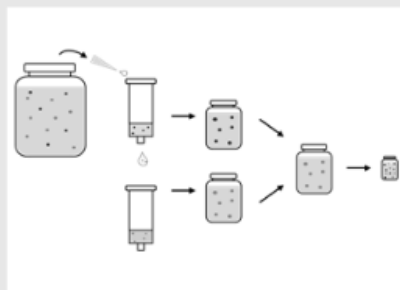
**The Ames Test has the best detection limits of all genotoxicity bioassays**

**Detection Limit further improved by miniaturization (approx. 5-fold)**

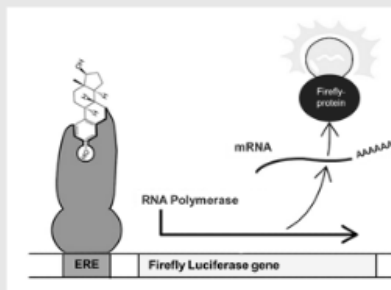
Parallel evaporation



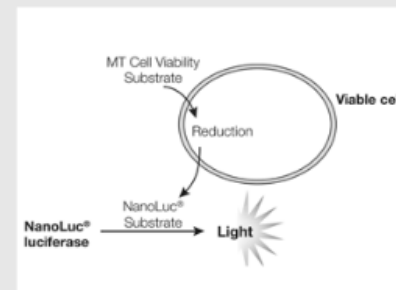
SSPE



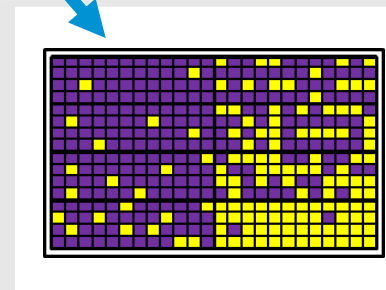
Endocrine activity  
(CALUX)



Cytotoxicity  
(RealTime-Glo)

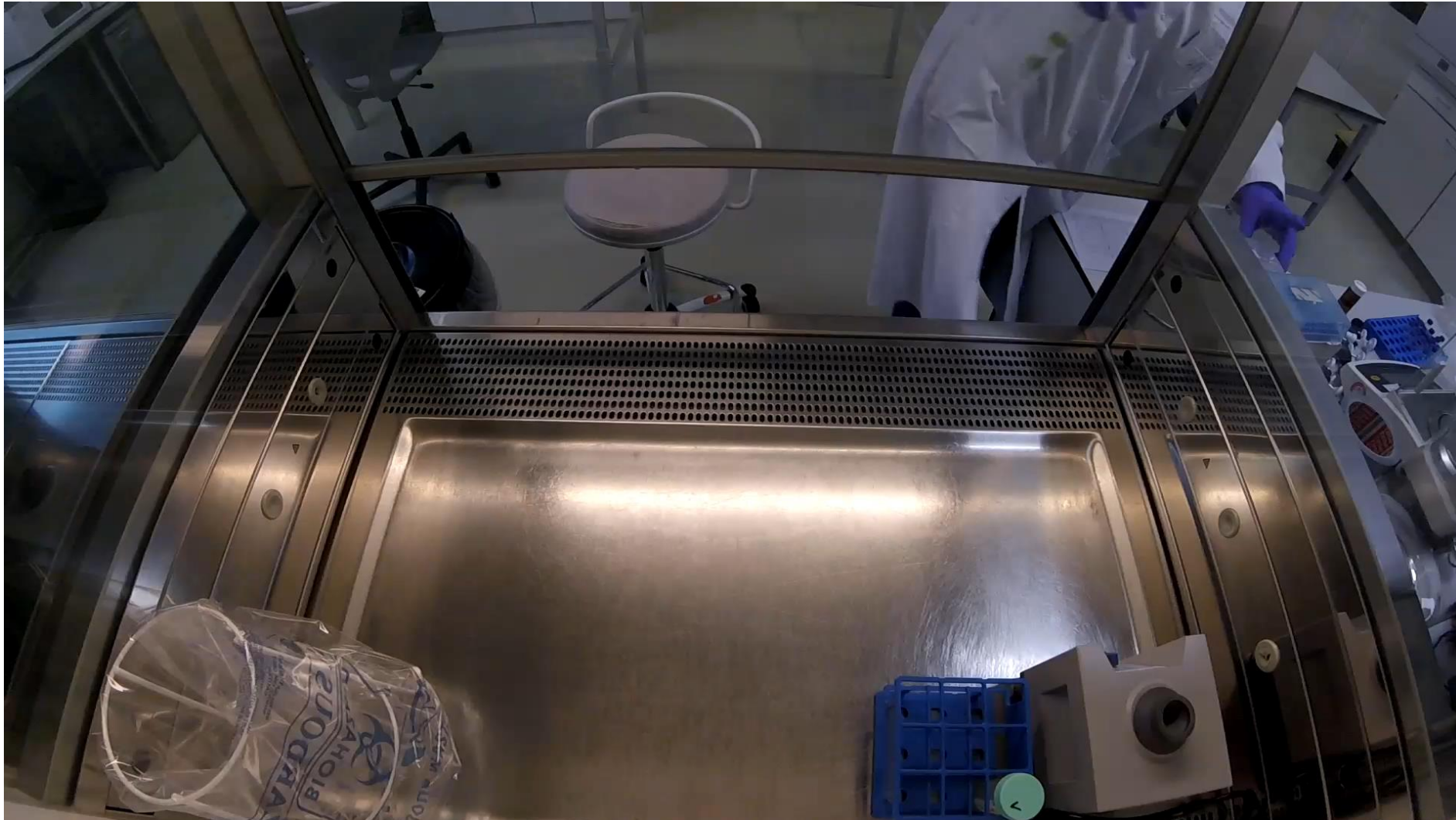


DNA-reactive genotoxicity  
(Miniaturized Ames)





# A sneak peek into our lab





Factors that influence the interpretation of Ames results:

- Contaminations during migration
- Contaminations during pre-concentration
- Reproducibility of the method
- Background revertants: too high/low
- Positive control response: too high/low
- Inhibiting effects
- Dose-response data
- Rules to “clear” false positive responses

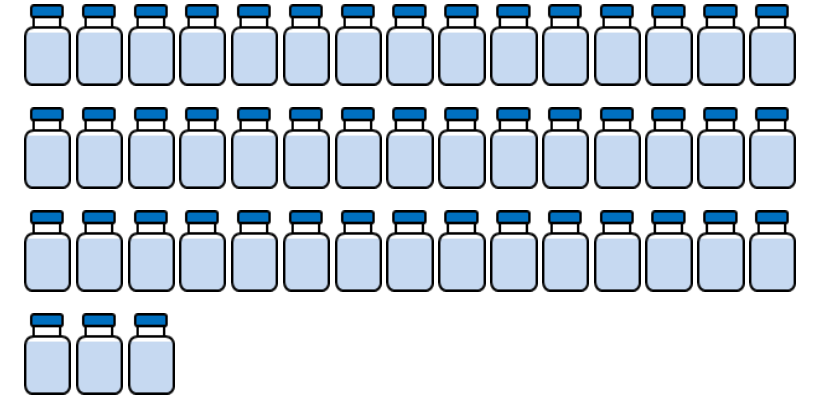


## To minimize the risk of contamination:

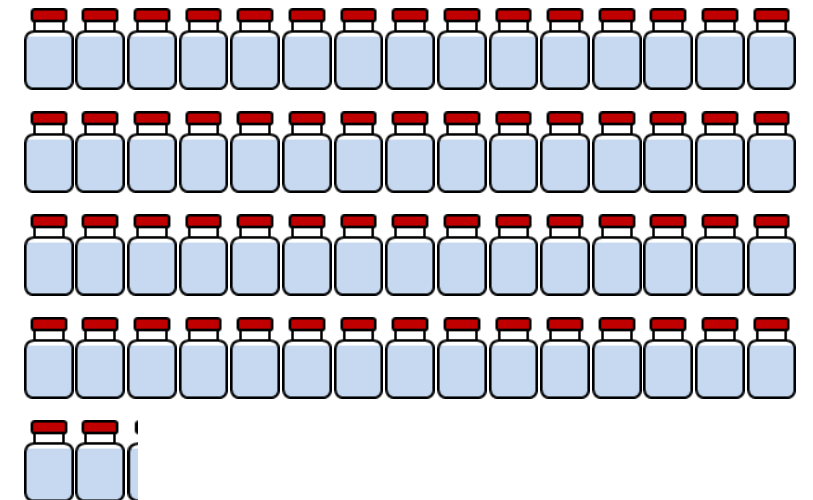
- High quality solvents (HPLC grade)
- Migration containers made out of glass
- Plastic parts covered with PTFE
- Glass vessels are rinsed with solvents before use
- Migration blanks are prepared

**Migrations blanks are rarely positive!**

**480  
Blanks**



**621  
Samples**





# Cross-contamination during parallel evaporation

## Syncore® Analyst set-up

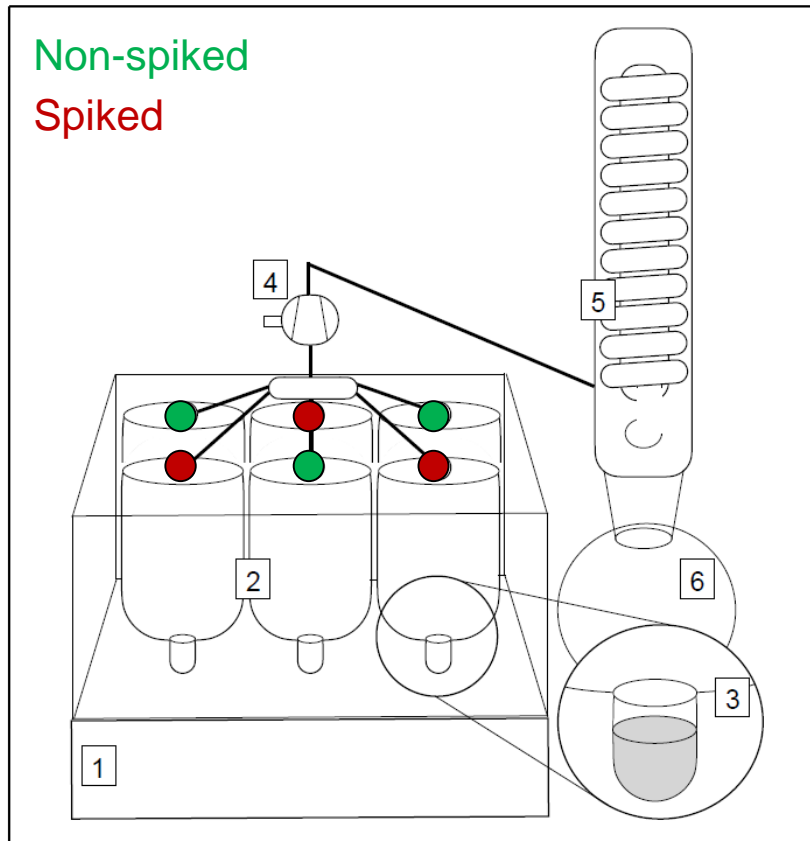


Figure 7: Schematic draft of the Büchi Syncore analyst setup. 1. Heatable and coolable shaking platform 2. Six glass flasks with appendix 3. Cooled appendix to avoid evaporation to dryness 4. Vacuum pump and control unit 5. Reflux cooler 6. Reservoir for evaporated and condensed food simulant.

Glass vessels are connected by **vacuum cover**:  
potential source of contamination?

To measure cross-contamination:

- 3 vessels: spiked with volatile model substances
- 3 vessels: non-spiked

Recovery in non-spiked vessels was determined by GC-MS

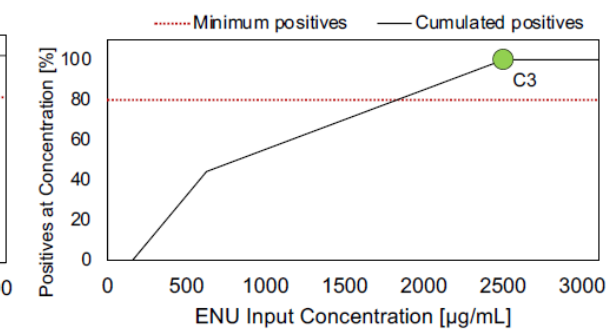
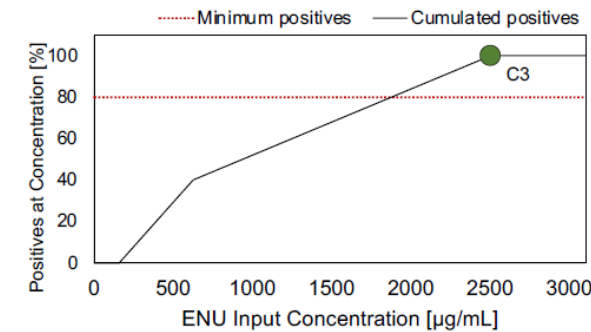
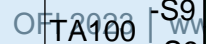
**Cross-contamination rate: <1 %**, but still detectable for some substances

**Are low cross contamination rates problematic?**



The logo consists of the lowercase letters 'ofi' in a bold, white, sans-serif font. To the right of the letters is a white circular arc. Inside this arc, the text 'FORTSCHRITT IN GUTEN HÄNDEN' is written in a smaller, white, sans-serif font, following the curve of the arc.

# INTRA





## Latest news: Multi-centre study organized by Xenometrix was published!

		Open	M1
K1	TA98	-S9	Green
		+S9	Red
	TA100	-S9	Green
		+S9	Green
K2	TA98	-S9	Green
		+S9	Green
	TA100	-S9	Green
		+S9	Green
C1	TA98	-S9	Red
		+S9	Red
	TA100	-S9	Green
		+S9	Red
C2	TA98	-S9	Green
		+S9	Green
	TA100	-S9	Green
		+S9	Green
P	TA98	-S9	Green
		+S9	Green
	TA100	-S9	Green
		+S9	Green

Mutation Research - Genetic Toxicology and Environmental Mutagenesis 893 (2024) 503718



Contents lists available at ScienceDirect

### Mutation Research - Genetic Toxicology and Environmental Mutagenesis

journal homepage: [www.elsevier.com/locate/gentox](http://www.elsevier.com/locate/gentox)



## Assessment of the performance of the Ames MPF™ assay: A multicenter collaborative study with six coded chemicals

Dimitrios Spiliotopoulos<sup>a,1,\*</sup>, Cécile Koelbert<sup>a</sup>, Marc Audebert<sup>b,c</sup>, Ilona Barisch<sup>d</sup>, Deborah Bellet<sup>e</sup>, Mathilde Constans<sup>b</sup>, Andreas Czich<sup>d</sup>, Francis Finot<sup>e</sup>, Véronique Gervais<sup>f</sup>, Laure Khoury<sup>b</sup>, Christian Kirchnawy<sup>g</sup>, Sachiko Kitamoto<sup>h</sup>, Audrey Le Tesson<sup>f</sup>, Laure Malesic<sup>e</sup>, Ryoko Matsuyama<sup>h</sup>, Elisa Mayrhofer<sup>g</sup>, Isabelle Mouche<sup>e</sup>, Birgit Preikschat<sup>d</sup>, Lukas Prielinger<sup>i</sup>, Bernhard Rainer<sup>i</sup>, Clémence Roblin<sup>f</sup>, Kerstin Wäse<sup>d</sup>

<sup>a</sup> Xenometrix AG, CH-4123 Allschwil, Switzerland

<sup>b</sup> PrediTox, 1 place Pierre Potier, 31100 Toulouse, France

<sup>c</sup> INRAE UMR1331 Toxalim, 180 chemin de Tournefeuille, 31300 Toulouse, France

<sup>d</sup> Genetic Toxicology, Preclinical Safety, Sanofi-Aventis Deutschland GmbH, 65926 Frankfurt am Main, Germany

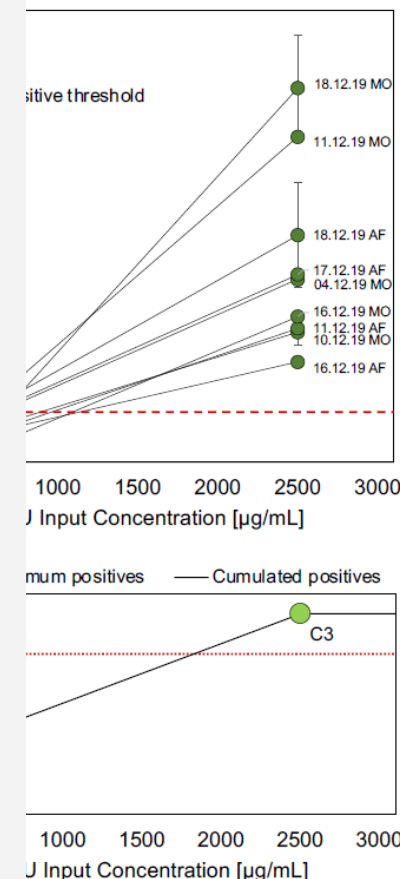
<sup>e</sup> GenEvolutionN, 2, 8 Rue de Rouen, 78440 Porcheville, France

<sup>f</sup> Servier Group, Non-Clinical Safety Department, F-45403 Orléans-Gidy, France

<sup>g</sup> OFI, Austrian Research Institute for Chemistry and Technology, Department for Microbiology and Cell Culture, Franz-Grill Straße 5, Objekt 213, 1030 Vienna, Austria

<sup>h</sup> Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., 1-98, Kasugadenaka 3-chome, Konohana-ku, Osaka, Japan

<sup>i</sup> Department of Applied Life Sciences, University of Applied Sciences, FH Campus Wien, Favoritenstraße 222, 1100 Vienna, Austria





# Calculation of the n-fold increase

Calculation of results of the miniaturized Ames test:

1. **Revertant wells** are counted  
Sample & Negative Control (DMSO, C-)  
(each analysed in triplicates)
2. **Mean + Std. dev.** are calculated from Triplicates
3. **Baseline:** Mean + Std. Dev. from C-
3. **Positive Threshold:** Baseline x 2
4. Comparison **mean sample vs. positive Threshold**  
Mean  $\geq$  pos. Threshold = positive  
Mean  $<$  pos. Threshold = negative

*Example:*

	Sample	C-
Revertants:	10, 6, 7	1, 2, 1
Mean:	7.6	1.3
Std. Dev.:	2.1	0.6

Baseline:  $1.3 + 0.6 = 1.9$   
Pos. Threshold:  $2 \times 1.9 = 3.8$

$7.6 > 3.8 \rightarrow$  sample genotoxic



# Calculation of the n-fold increase

## Calculation of results of the miniaturized Ames test:

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Mean < pos. Threshold = negative

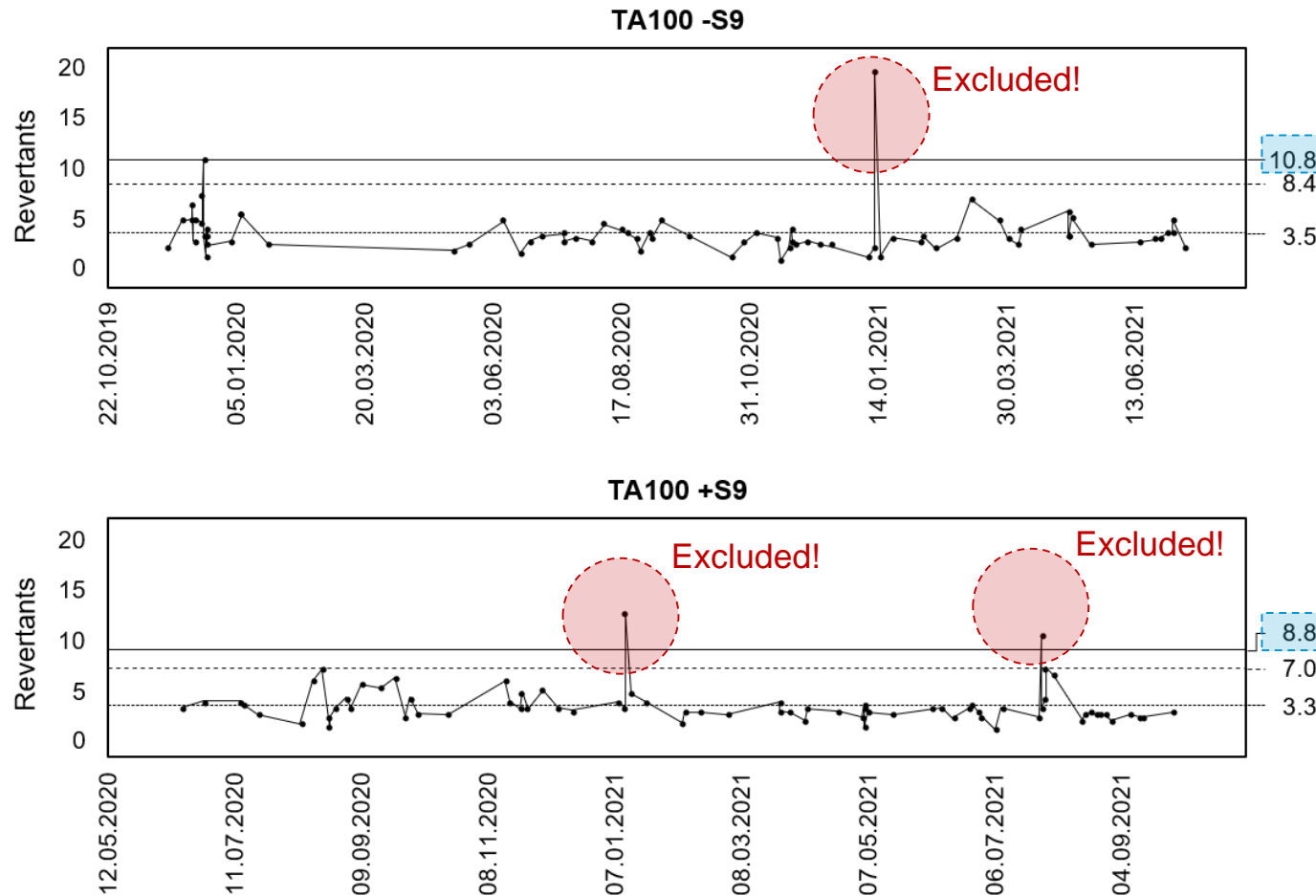
### Example:

	Sample	C-	5
Revertants:	10, 6, 7	1, 2, 1	
Mean:	7.6	<del>1.3</del>	2.6
Std. Dev.:	2.1	<del>0.6</del>	2.1
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# Quality control charts to track background levels

## Quality control chart - negative control



Problem: **High backgrounds** / negative control responses could **cover positive results**.

**Upper limits** for background revertants were determined based on historical data.

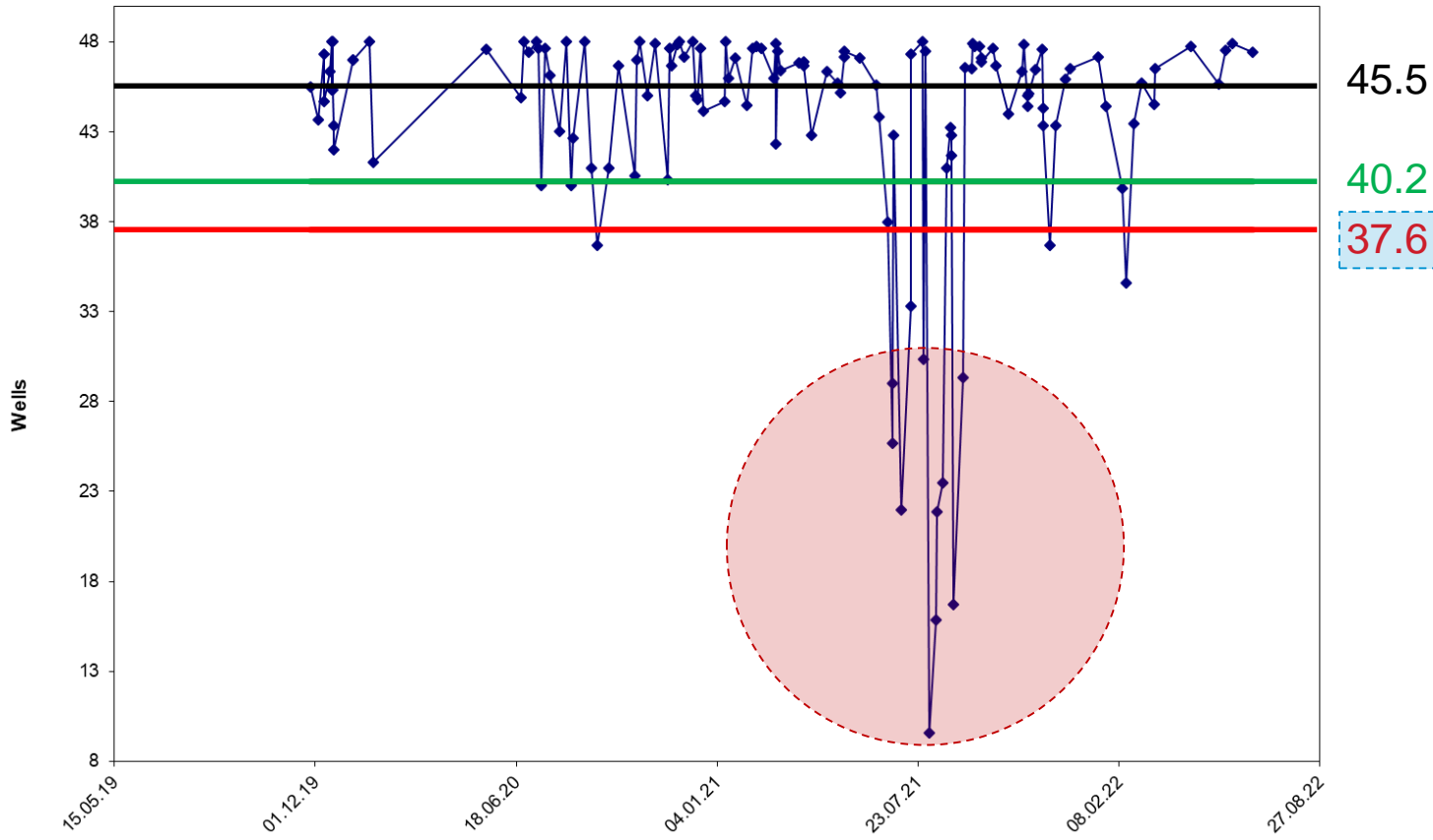
Assays **crossing** these **thresholds** are **excluded** from the evaluation.



# Low positive control responses indicate poor assay performance

## Quality control chart - positive control

TA98 –S9



Low positive control responses trigger false negative results.

Lower limits for positive control revertants were determined based on historical data to exclude unsuitable assays.

### *Practical example:*

- Summer 2021: A series of assays did not work (TA98 –S9).
- Problems with o/n culture identified as cause (OD & dilution).
- Cultivation procedure adapted.

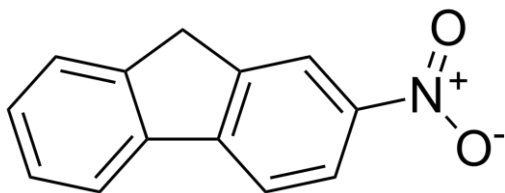


# Inhibiting effects cause insufficient recovery of the internal standard

To check for inhibiting effects from the FCM sample:  
Spiking with mutagenic reference substances

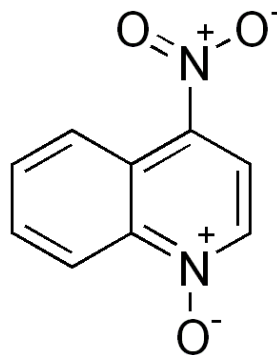
TA98 –S9

2-Nitrofluorene



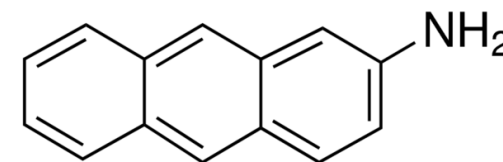
TA100 –S9

4-Nitroquinoline-N-oxide



TA98 / TA100 +S9

2-Aminoanthracene



Inhibition by:

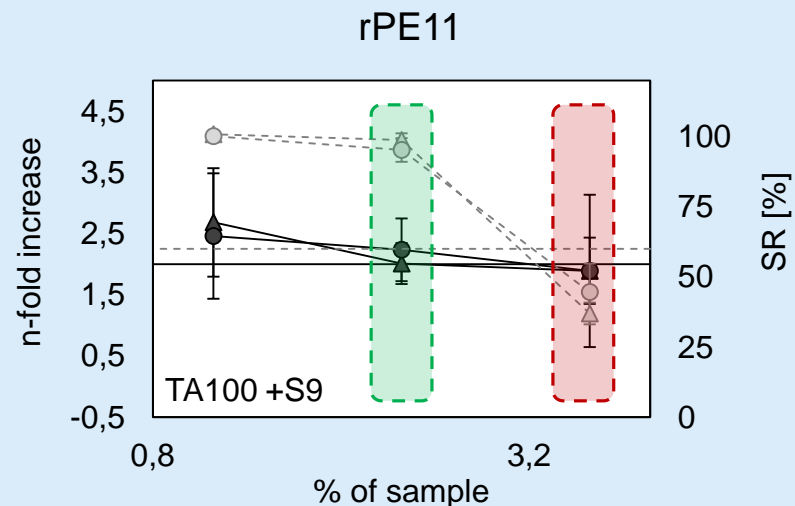
Bacteriotoxic effects  
Bacteriostatic effects

Inhibition of enzymatic function (+S9)  
Reduction of bioavailability



# Inhibiting effects cause insufficient recovery of the internal standard

To check for inhibiting effects from the FCM sample:  
**Spiking with mutagenic reference substances**



- **Undiluted**: inhibiting, non-DNA-reactive
- **1:2 dilution**: not inhibiting, DNA-reactive

Only if the **internal standard is recovered sufficiently**:  
**Assay is valid & can be used for data interpretation!**

Migrate 1: ● n-fold increase ○ SR [%]  
Migrate 2: ▲ n-fold increase △ SR [%]

— 2-fold increase    - - - 60 % SR

Inhibition by:

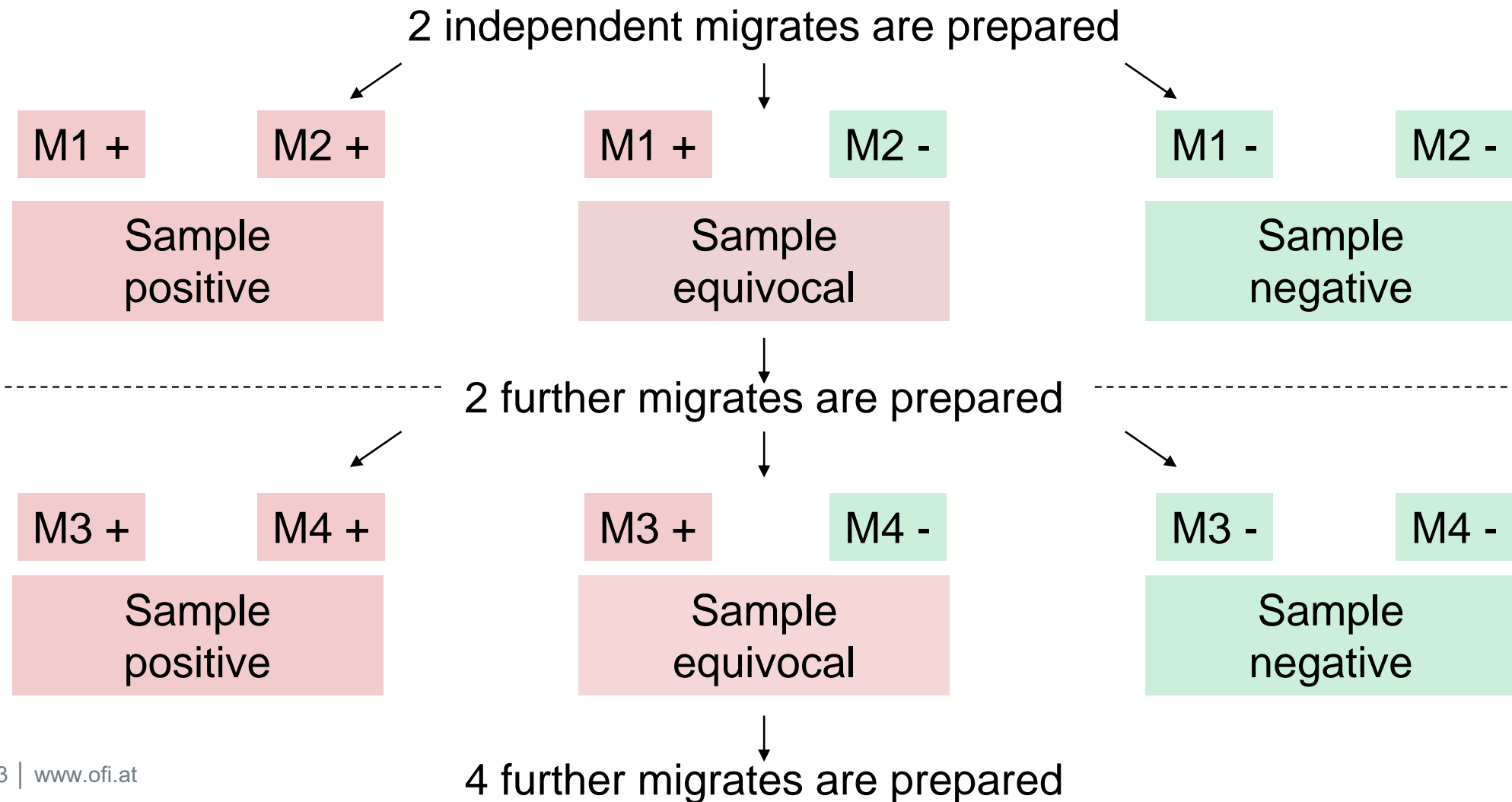
Bacteriotoxic effects  
Bacteriostatic effects

Inhibition of enzymatic function (+S9)  
Reduction of bioavailability



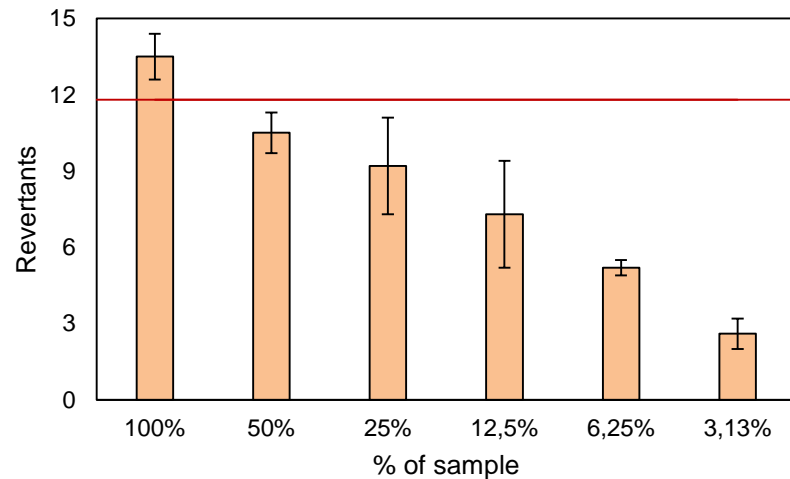
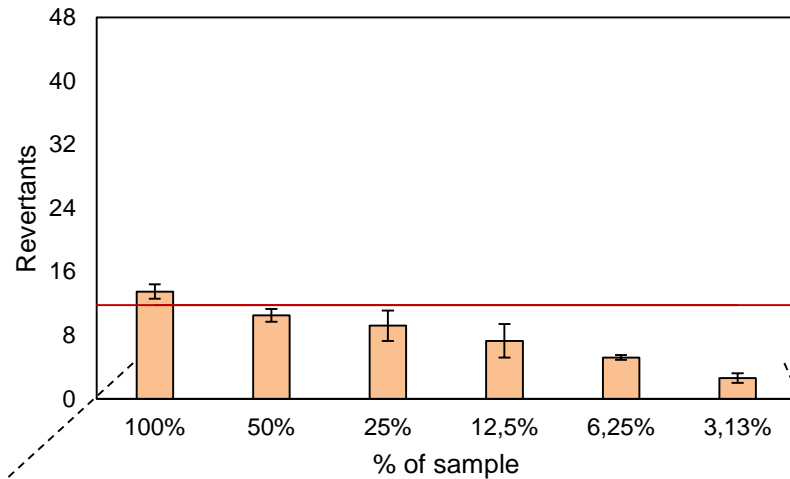
# Rule: „3 negative results can clear 1 positive“

Strategy developed with Prof. David Kirkland

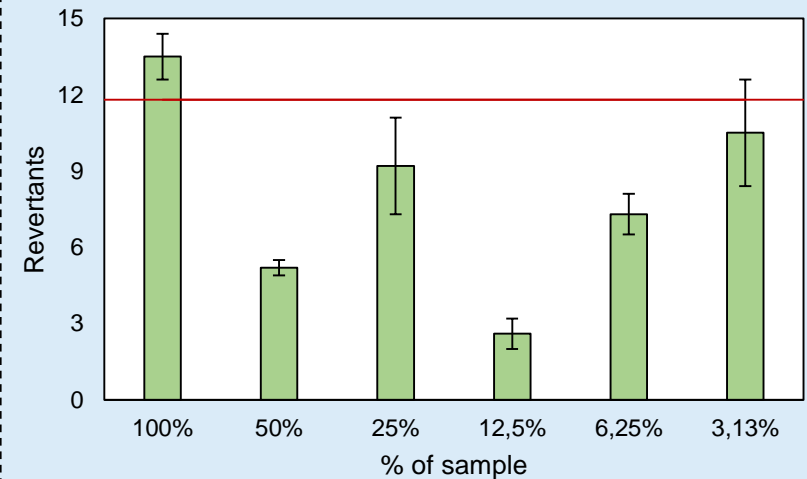
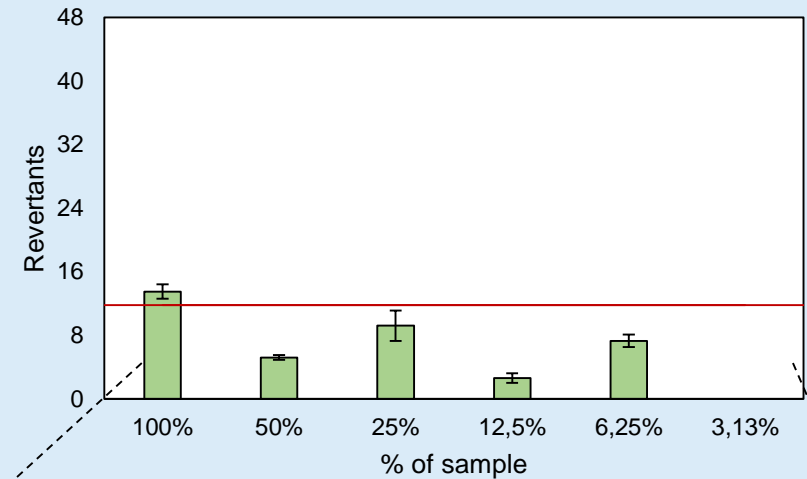




# Dose response relationship supports positive results



Supports a true positive



Supports a false positive



# Correct data interpretation is key!

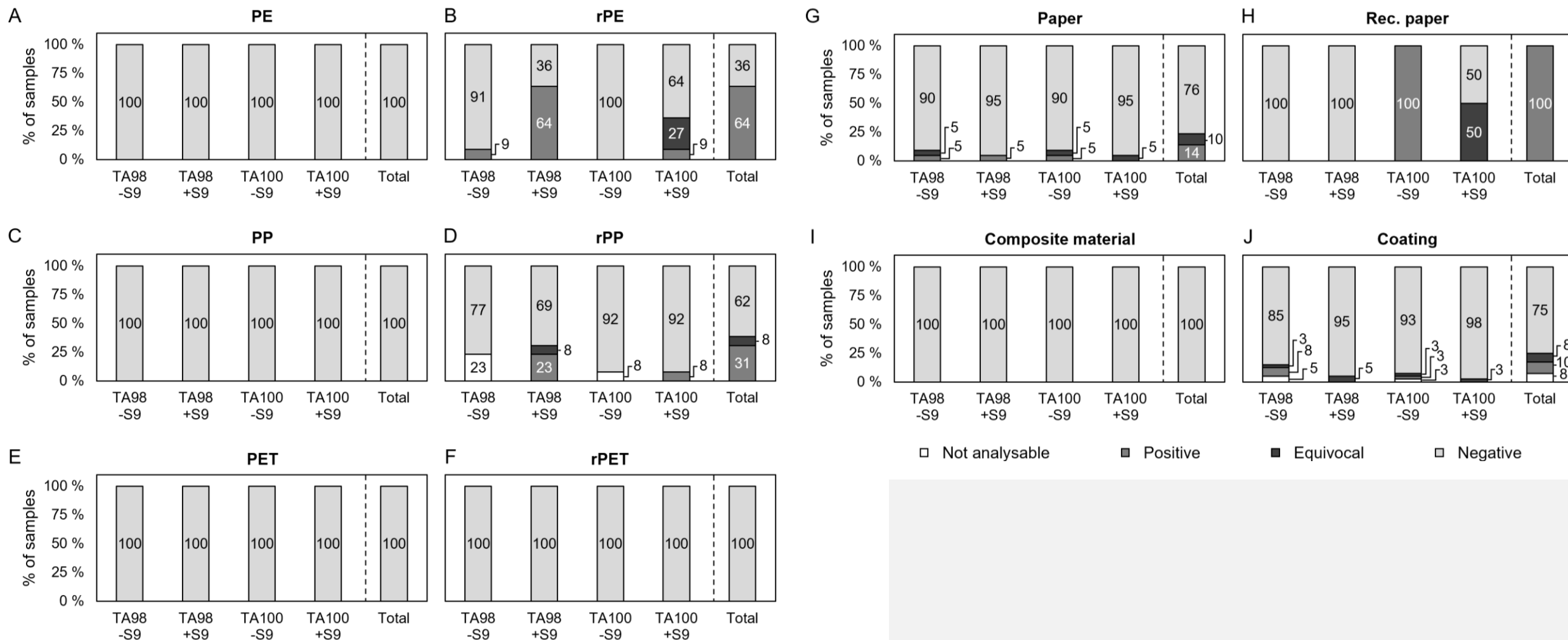
Factors that influence the interpretation of Ames results:

- ✓ Contaminations during migration
- ✓ Contaminations during pre-concentration
- ✓ Reproducibility of the method
- ✓ Background revertants: too high/low
- ✓ Positive control response: too high/low
- ✓ Inhibiting effects
- ✓ Dose-response data
- ✓ Rules to “clear” false positive responses

Processes were optimized  
& standardized

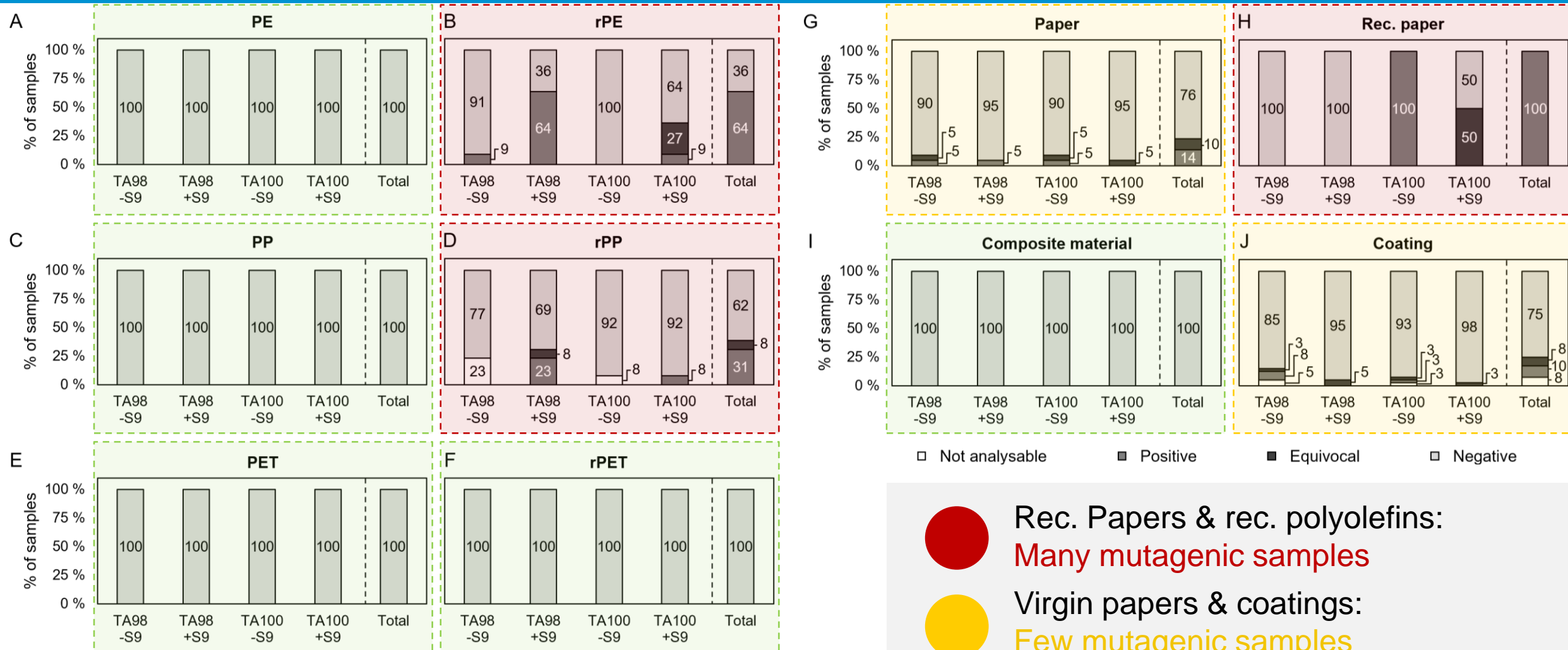


# Mutagenicity: Screenings results FCM samples





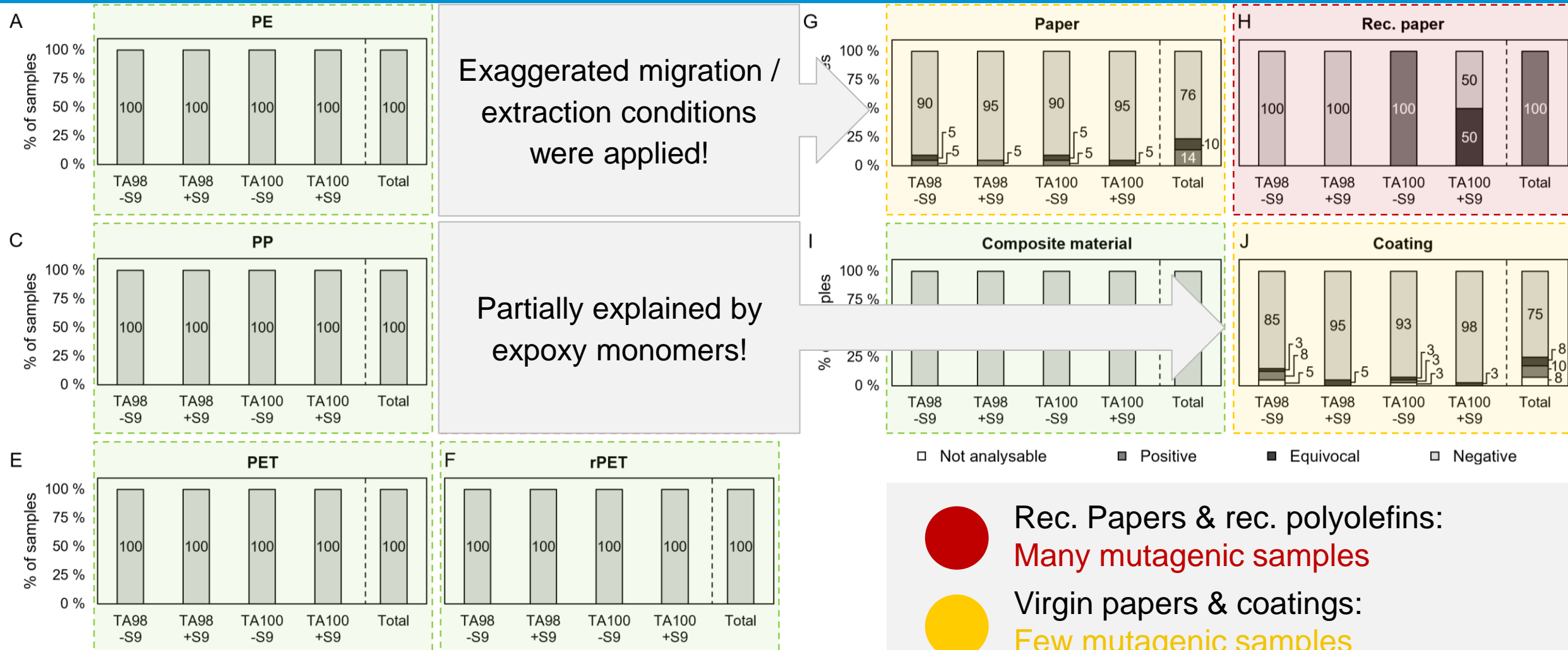
# Mutagenicity: Screenings results FCM samples



- Rec. Papers & rec. polyolefins:  
Many mutagenic samples
- Virgin papers & coatings:  
Few mutagenic samples
- Virgin plastics, composites & rec. PET:  
No mutagenic samples



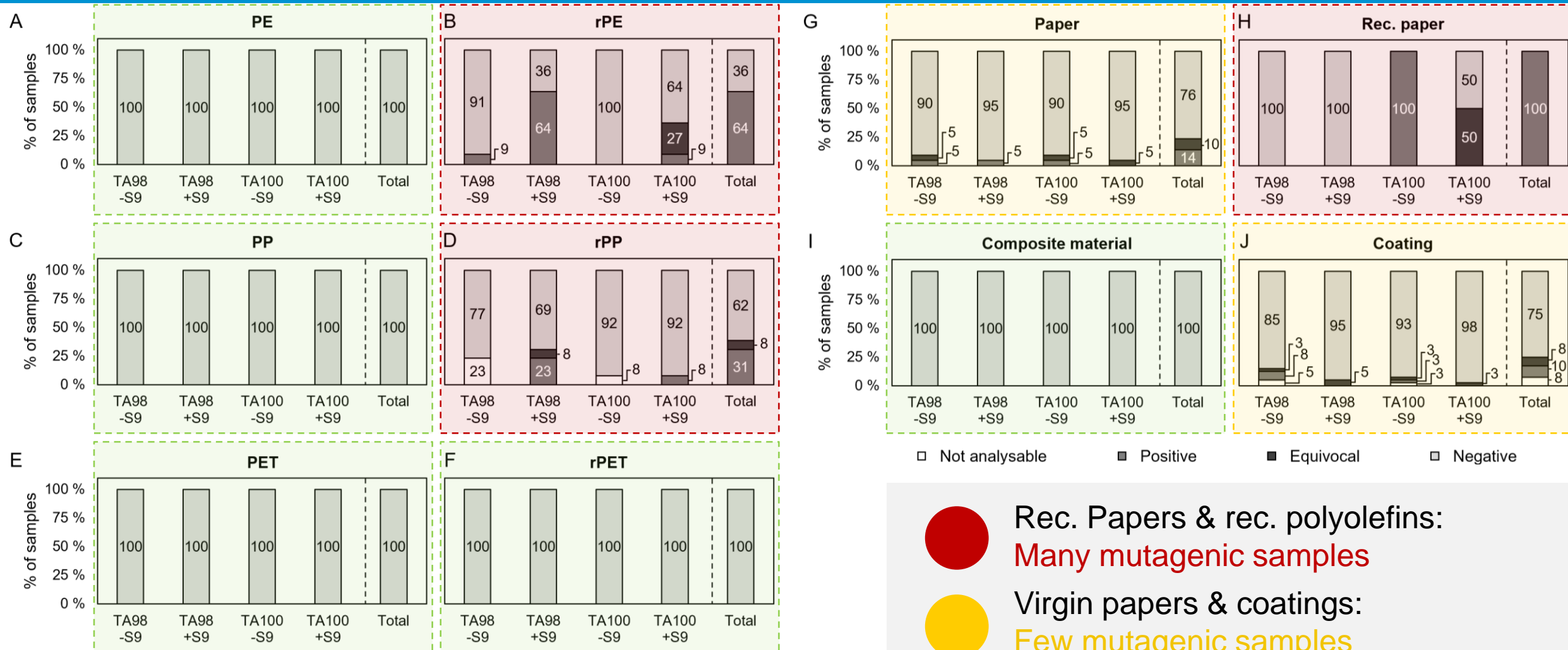
# Mutagenicity: Screenings results FCM samples



- Rec. Papers & rec. polyolefins: **Many mutagenic samples**
- Virgin papers & coatings: **Few mutagenic samples**
- Virgin plastics, composites & rec. PET: **No mutagenic samples**



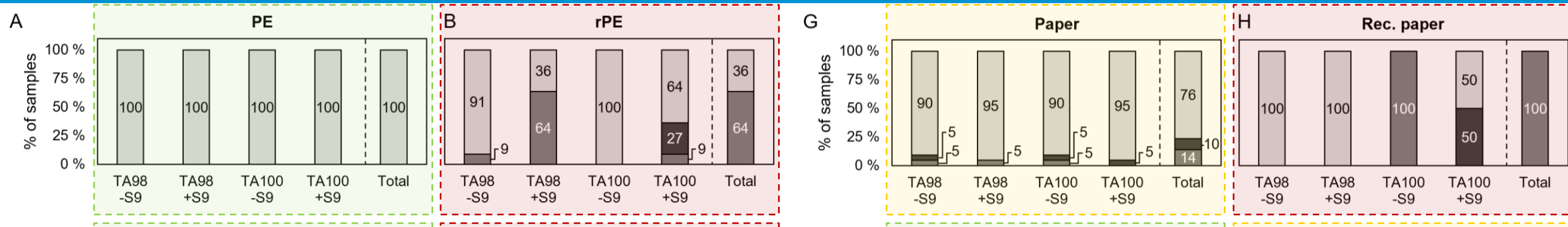
# Mutagenicity: Screenings results FCM samples



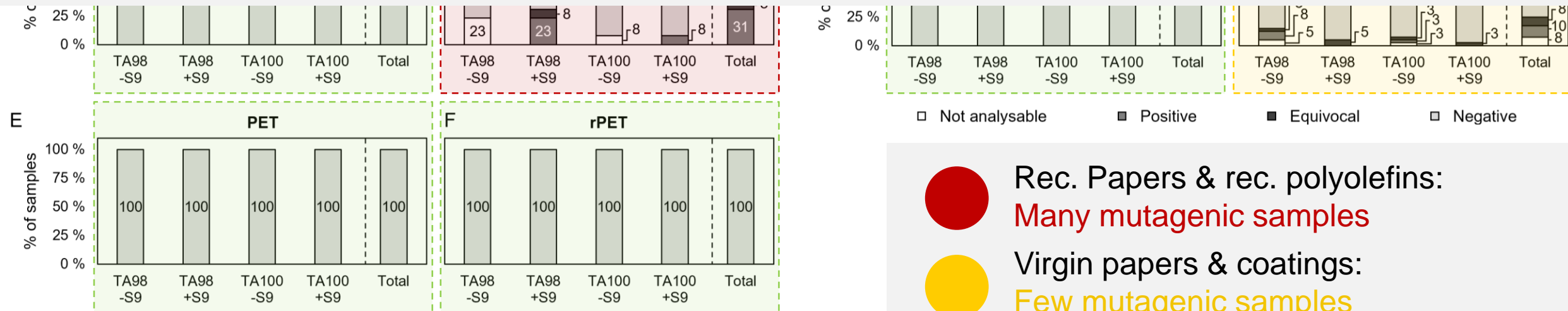
- Rec. Papers & rec. polyolefins:  
**Many mutagenic samples**
- Virgin papers & coatings:  
**Few mutagenic samples**
- Virgin plastics, composites & rec. PET:  
**No mutagenic samples**



# Mutagenicity: Screenings results FCM samples



Food contact materials score mainly non-mutagenic!



- Rec. Papers & rec. polyolefins: **Many mutagenic samples**
- Virgin papers & coatings: **Few mutagenic samples**
- Virgin plastics, composites & rec. PET: **No mutagenic samples**





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