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Performance of miniaturized Ames assays indicate high predictive power for mutagenicity

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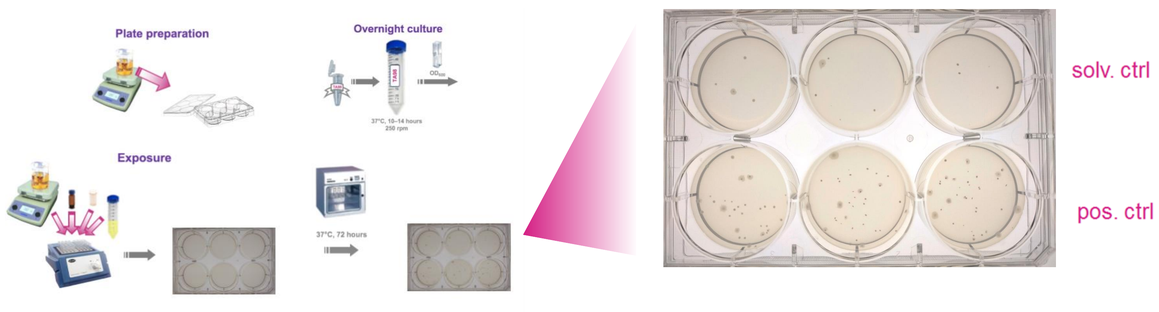
Introduction

The Ames assay is a bacterial reverse gene mutation test that has been a cornerstone of mutagenicity assessment. The emphasis now is on developing miniaturized versions of the traditional Ames test to require **less chemicals, reagents, and liver microsomal S9 fraction**, thus reducing the number of test animals needed and to better comply with 3R principles. **Miniaturized Ames** assay versions promote **high throughput testing** of multiple samples in the course of compound screening and facilitate the **early exclusion of genotoxic agents** during the product development process. Existing experimental data shed light on a **high concordance** between results gained with miniaturized Ames tests and the traditional Petri dish-based method, yet further testing is required to corroborate these findings. This current poster aims at providing a more detailed picture on this topic with an extended testing and data comparison paradigm.

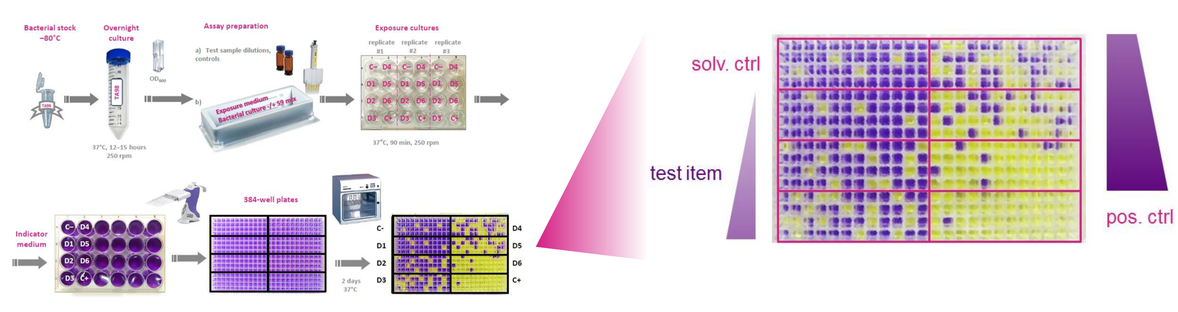
Methods

The compounds tested in the scope of this study were selected based on the OECD DRP list [1], namely chemicals with false negative, false positive or equivocal categorization of the Ames MPF system in comparison with the Petri dish-based test. In silico prediction of mutagenic potential of the substances was assessed using the open-source ToxTree web-based tool [2]. Data from the NTP database [3] was extracted for further comparison with Petri dish-based assay results. The compounds presented on this poster were tested in two different **parallel running miniaturized Ames assay systems** using the same O/N culture for both tests. For the metabolic activation rat or hamster liver S9 was applied in the **MicroAmes6** and **Ames MPF** assays, in a concentration of 10% and 30%, respectively.

The **Xenometrix MicroAmes6** is an agar-based miniaturized Ames test.



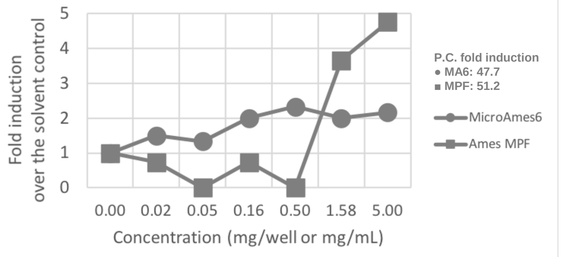
The **Xenometrix Ames MPF™** is a microplate fluctuation assay.



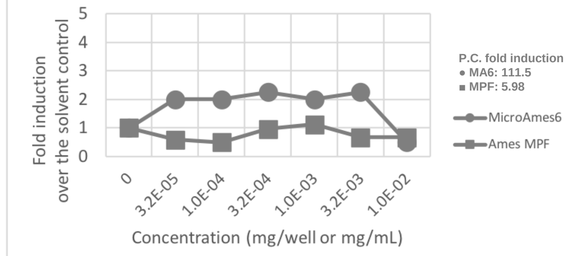
Results

TA98

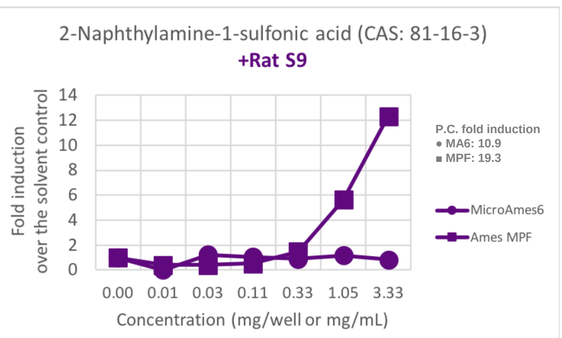
m-Phenylenediamine (CAS: 108-45-2)
without metabolic activation



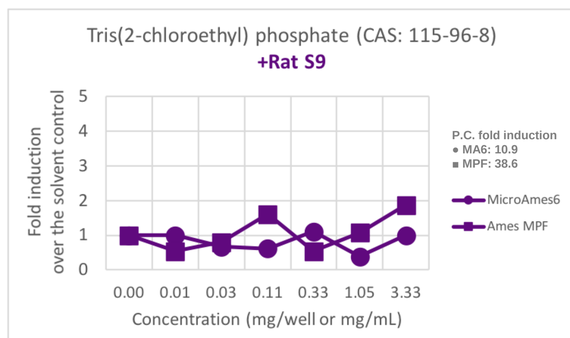
Benzo[a]pyrene (CAS: 50-32-8)
without metabolic activation



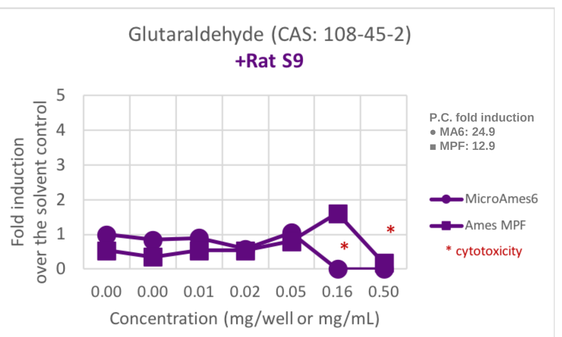
2-Naphthylamine-1-sulfonic acid (CAS: 81-16-3)
+Rat S9



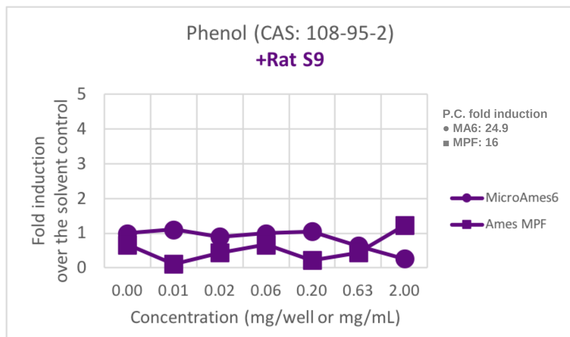
Tris(2-chloroethyl) phosphate (CAS: 115-96-8)
+Rat S9



Glutaraldehyde (CAS: 108-45-2)
+Rat S9

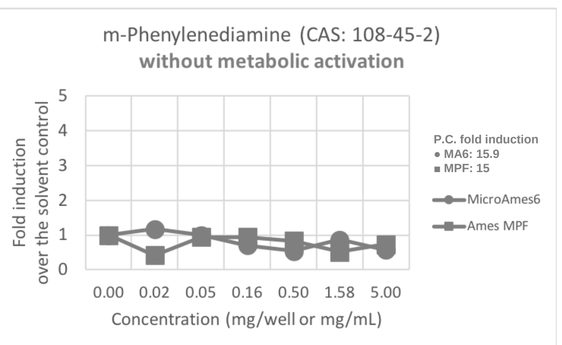


Phenol (CAS: 108-95-2)
+Rat S9

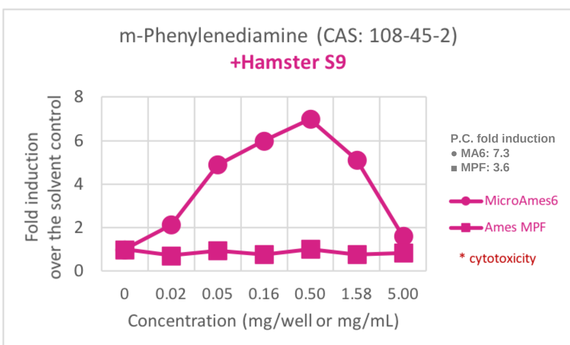


TA100

m-Phenylenediamine (CAS: 108-45-2)
without metabolic activation

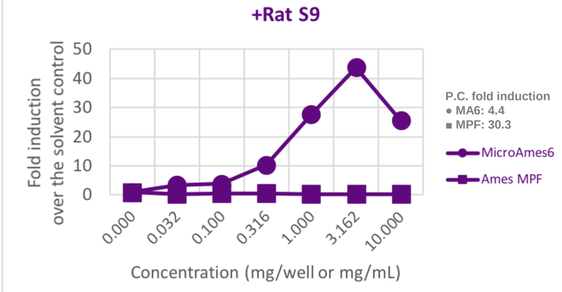


m-Phenylenediamine (CAS: 108-45-2)
+Hamster S9

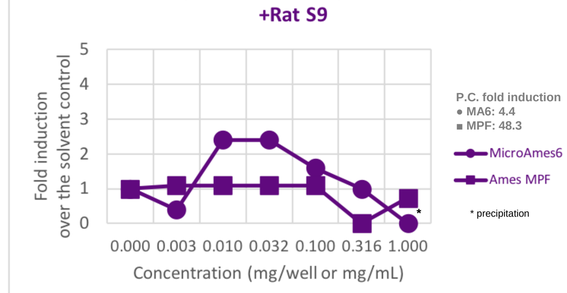


TA1535

Acetaldehyde oxime (CAS: 107-29-9)
+Rat S9

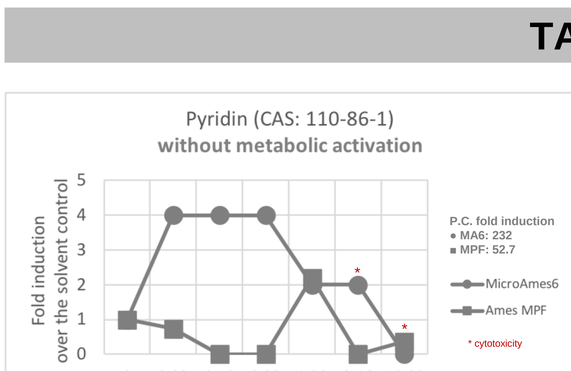


2-Acetylaminofluorene (CAS: 53-96-3)
+Rat S9

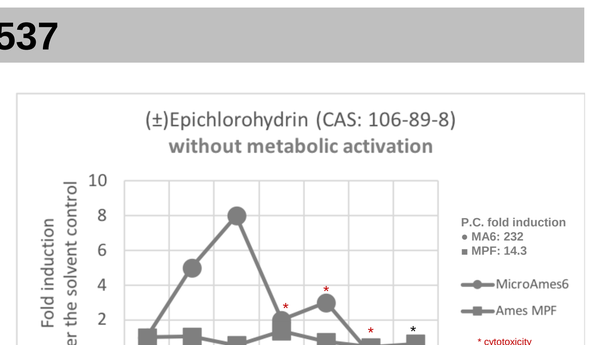


TA1537

Pyridin (CAS: 110-86-1)
without metabolic activation



(±)Epichlorohydrin (CAS: 106-89-8)
without metabolic activation



Number of spontaneous revertant colonies of the TA1537 strain in the MicroAmes6 is low (equal to or lower than 1 colony per well). This was taken into consideration during the interpretation of the results.

Summary of the results and comparison with other data

Compound	CAS-Number	Structural alert based on in silico prediction	Strain	Metabolic activation	Ames MPF category in OECD DRP	MicroAmes6	Ames MPF	NTP database
m-Phenylene-diamine	108-45-2	POS	TA98	Without S9	FN	Weak POS	POS	NEG
			TA100	Without S9	N.A.	NEG	NEG	NEG
			TA100	With Hamster S9	FN	POS	NEG	POS
Benzo[a]pyrene	50-32-8	POS	TA98	Without S9	FP	Weak POS	NEG	NEG
2-Naphthylamine-1-sulfonic acid	81-16-3	NEG	TA98	With Rat S9	FP	NEG	POS	N.A.
Tris(2-chloroethyl) phosphate	115-96-8	POS	TA98	With Rat S9	FP	NEG	NEG	NEG
Glutaraldehyde	111-30-8	POS	TA98	With Rat S9	FP	NEG	NEG	NEG
Phenol	108-95-2	NEG	TA98	With Rat S9	FP	NEG	NEG	NEG
Acetaldehyde oxime	107-29-9	NEG	TA1535	With Rat S9	FN	POS	NEG	POS
2-Acetylamino-fluoerene	53-96-3	POS	TA1535	With Rat S9	FP	Weak POS	NEG	N.A.
Pyridin	110-86-1	NEG	TA1537	Without	EQ	EQ	NEG	NEG
(±)Epichlorohydrin	106-89-8	POS	TA1537	Without S9	FN	EQ	NEG	NEG

POS = Positive; Weak POS = Weak Positive; NEG = Negative; EQ = Equivocal; FN = False Negative; FP = False Positive; N.A. = Not Available

Conclusion

Our results indicate that there are characteristic differences in the performance of the two miniaturized Ames test systems: in the case of some chemicals the 6-well plate-based MicroAmes6 results in a positive outcome of mutagenicity, whereas in cases of other chemicals, the liquid microplate fluctuation-based Ames MPF gives a positive answer. This finding can be explained by the conceptual differences between the two assays, one being an agar-based plate incorporation test as opposed to the other, which is a liquid system. It is also important to highlight the difference between the percentage of S9 applied in the two miniaturized systems, which can also account for the differential patterns in the acquired results. Interestingly, the structure-based in silico predictions only partially align with the in vitro outcomes in our study. The overall high concordance between the miniaturized and Petri dish-based Ames test results suggests that the miniaturized Ames assay variations can be designated as a highly reliable test method in mutagenicity assessment. The results presented in the scope of this work highlight an appropriate sensitivity and specificity of the miniaturized Ames test versions rendering them applicable across industries.

References:
[1] OECD Draft Detailed Review Paper on the miniaturised versions of the bacterial reverse gene mutation test
[2] Patlewicz G, Jeliazkova N, Safford RJ, Worth AP, Aleksiev B. (2008) An evaluation of the implementation of the Cramer classification scheme in the Toxtree software. SAR QSAR Environ Res. ;19(5-6):495-524.
[3] National Toxicology Program (NTP) coordinated by United States Department of Health and Human Services

The **AmesMPF** and **MicoAmes6** are proprietary miniaturised Ames assays developed by Xenometrix AG.
Check out our homepage **xenometrix.ch** for further information, and follow us on social media.