

Miniaturized Ames test systems for nitrosamine mutagenicity screening: A systematic evaluation of miniaturized Ames test formats as sustainable alternatives to conventional genotoxicity testing

Csaba Boglári^{1*}, Cécile Koelbert¹¹Xenometrix AG, Gewerbestrasse 25, 4123 Allschwil, Switzerland

* Corresponding author (cbo@xenometrix.ch)

Introduction

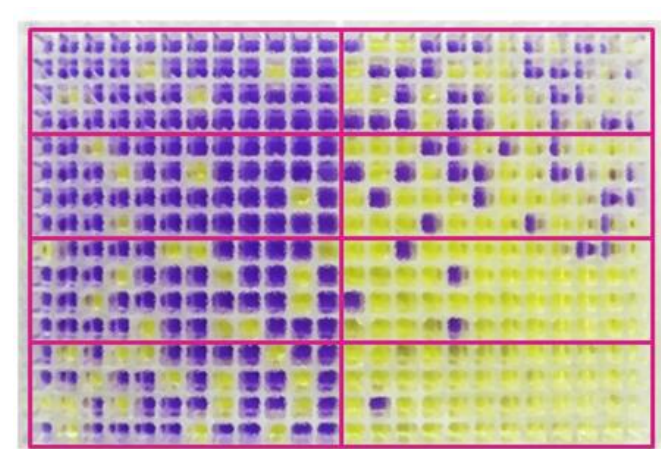
N-nitrosamines are potent mutagens and carcinogens that can contaminate pharmaceuticals, food packaging, and environmental samples. Because of their health risks, testing them for mutagenicity is essential, and the Ames test has long been the standard method for this purpose. Recent studies on compounds like NDMA and NDEA have clarified which experimental conditions most affect assay sensitivity, helping improve detection accuracy [1]. Nitrosamines can also form unintentionally—for example, during water treatment, when secondary amines react under oxidative conditions to create mutagenic by-products [2]. Their presence in pharmaceuticals is a particular concern, prompting regulators like the FDA and EMA to tighten oversight of manufacturing processes. Current guidance recommends using the Enhanced Ames Test (EAT), which calls for higher S9 concentrations and the inclusion of hamster liver S9 alongside rat liver S9 [3,4].

Highlights

- Enhanced Ames Test (EAT) conditions implemented in miniaturized Ames assay context
- High agreement between miniaturized Ames test formats
- Miniaturized Ames assays can detect positive Nitrosamines at lower concentrations compared to the Petri dish-based test
- Adjusted protocol for volatile Nitrosamine compounds
- 3R benefits: less S9, less compound, and minimal plastic waste

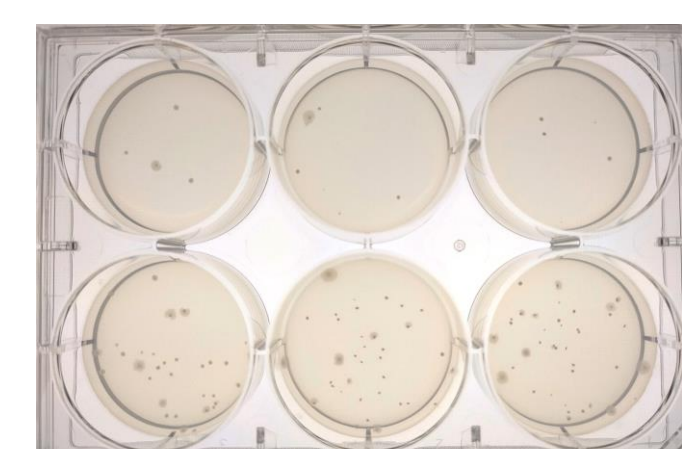
Methods: parallel running miniaturized Ames tests from the same overnight bacterial culture

Ames MPF™



- Miniaturized Ames test in microplate fluctuation format
- Ames tester strains: TA100, TA1535, E.coli uvrA[pKM101]
- Water was used as solvent, unless solubility was an issue → DMSO
- Metabolic activation: 30% Hamster Liver S9 (in accordance with EAT)
- Input bacterial cell density: 10⁸ cells per mL
- 25x concentrated stock solution of Nitrosamine test compounds
- Pre-incubation for 90 minutes – concentration was adjusted to have the same test compound concentration between the two miniaturized systems

Pre-incubation MicroAmes6

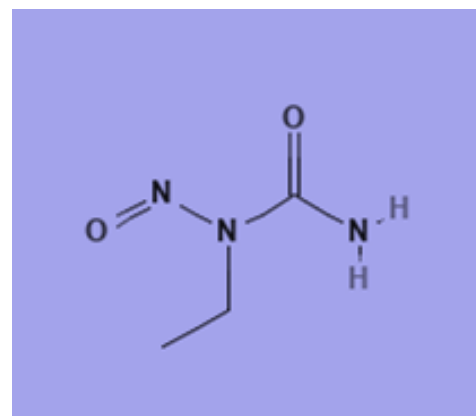


- Miniaturized Ames test in 6-well agar plate format with a pre-incubation step
- Ames tester strains: TA100, TA1535, E.coli uvrA[pKM101]
- Water was used as solvent, unless solubility was an issue → DMSO
- Metabolic activation: 30% Hamster Liver S9 (in accordance with EAT)
- Input bacterial cell density: 10⁷ cells per mL
- 50x concentrated stock solution of Nitrosamine test compounds
- Pre-incubation for 30 minutes – concentration was adjusted to have the same test compound concentration between the two miniaturized systems

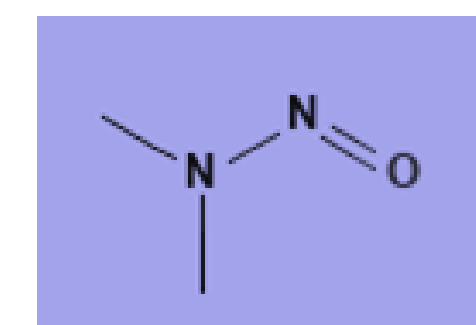
Results

Performance of the miniaturized Ames assays in Nitrosamine testing

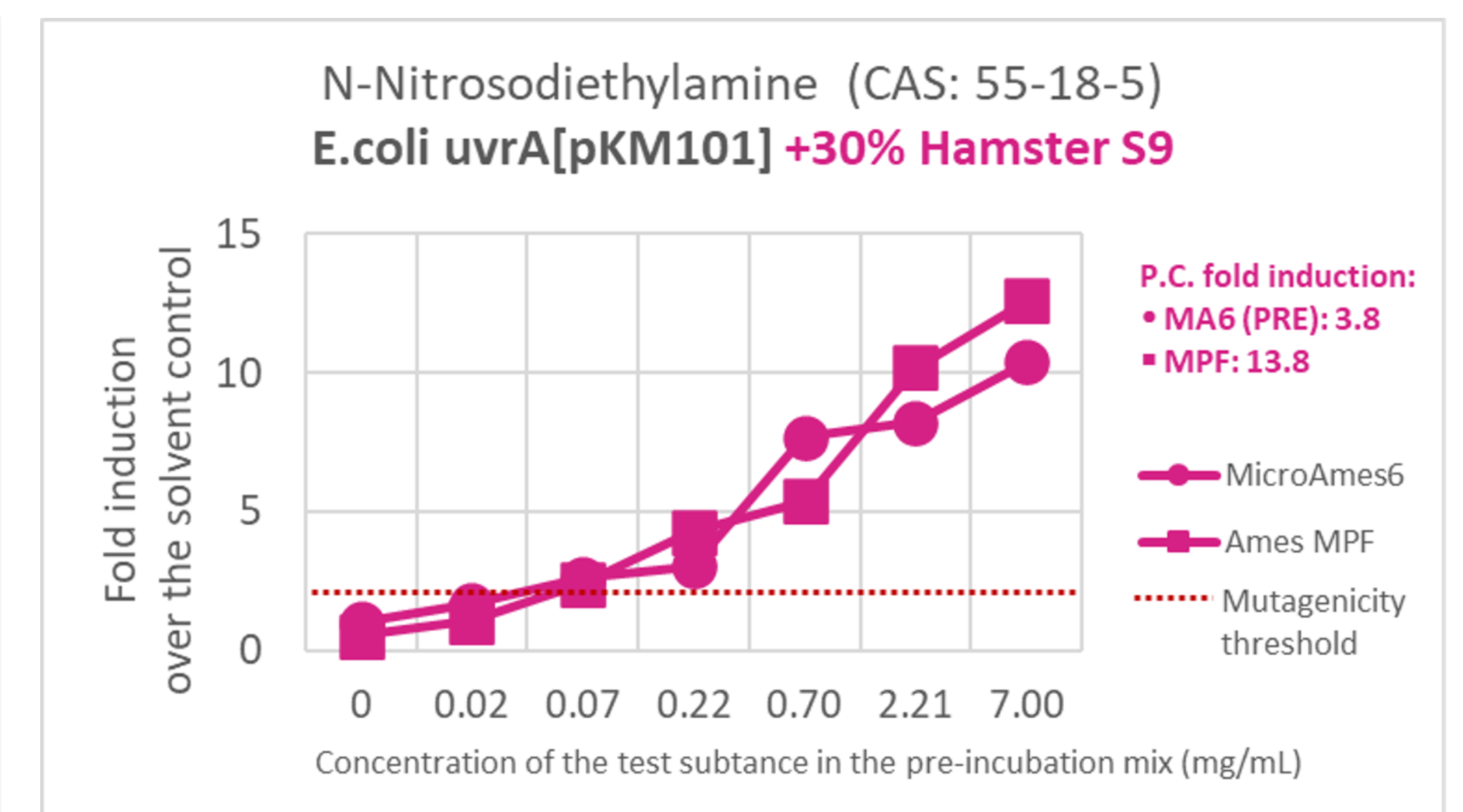
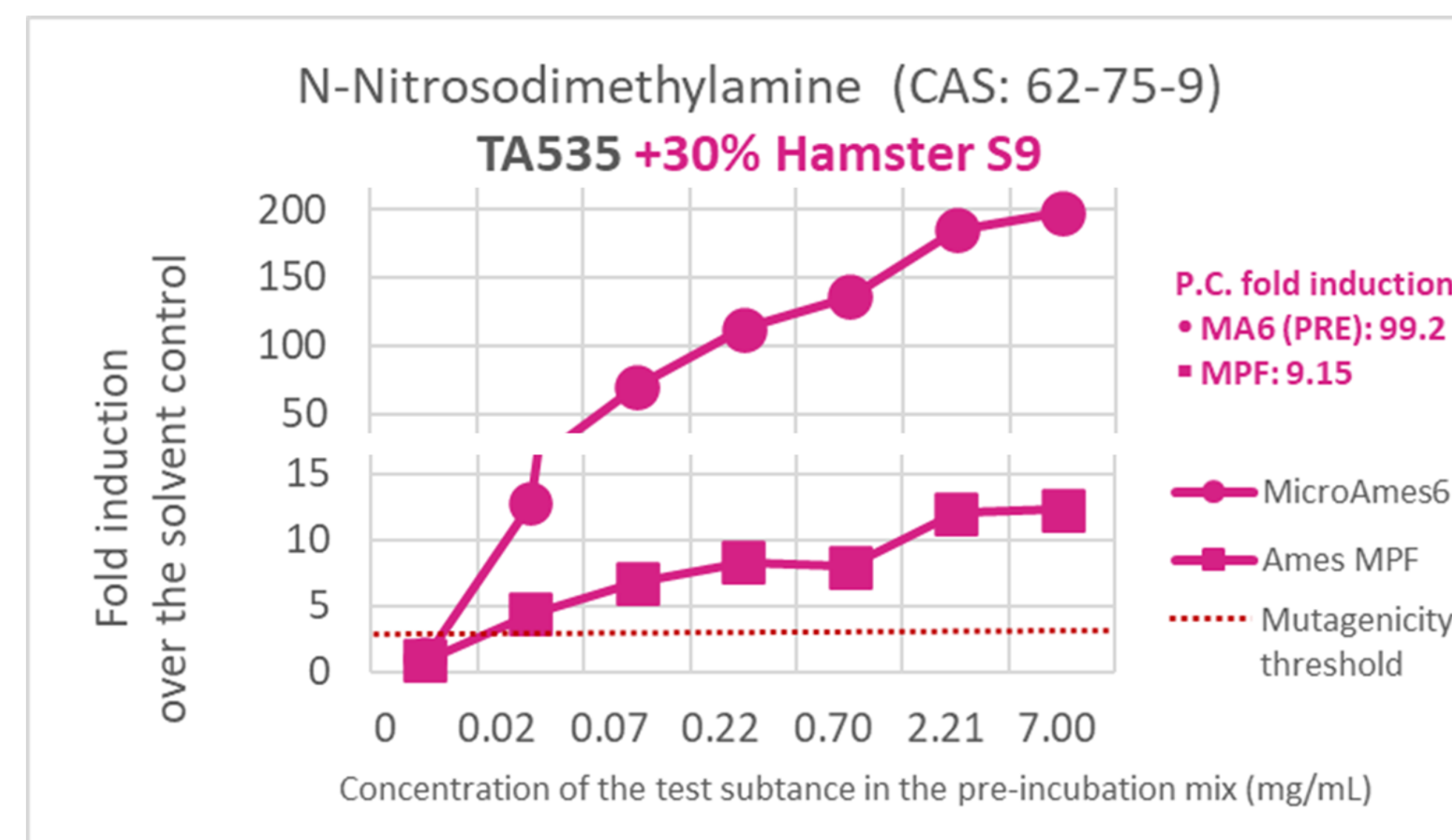
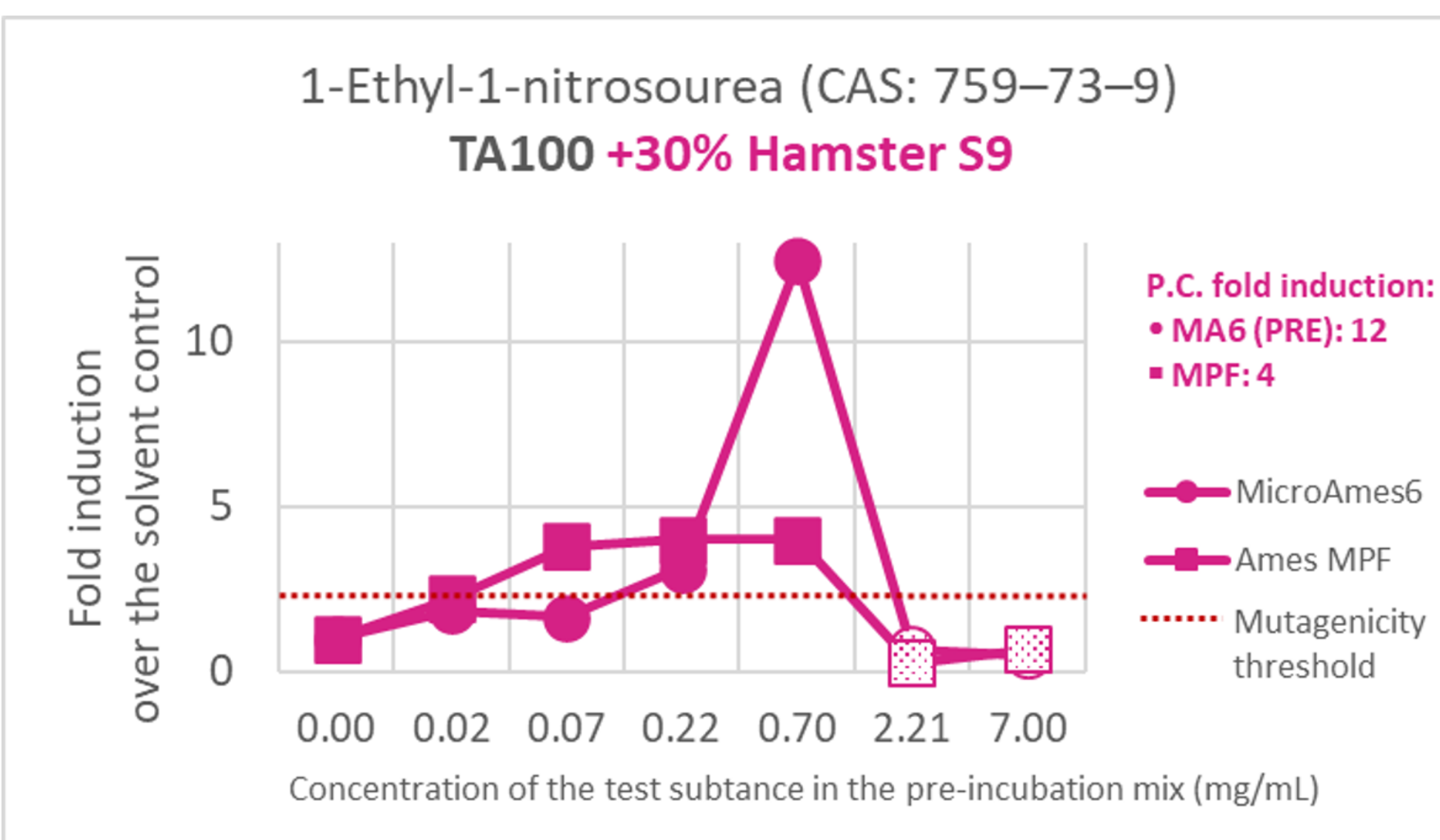
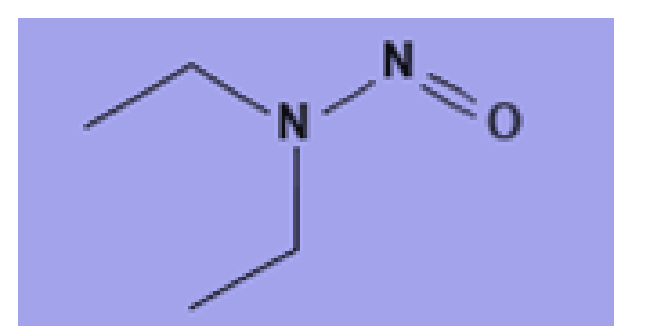
1-Ethyl-1-Nitrosourea (ENU)



N-Nitrosodimethylamine (NDMA)



N-Nitrosodiethylamine (NDEA)



Volatility of small Nitrosamines

Small nitrosamines are prone to evaporation

Classification by boiling point — <100°C very volatile · 75–250 volatile · 250–390 semivolatile · >390 nonvolatile.

COMPOUND	CAS	MW (G/MOL)	BOILING POINT	VOLATILITY
N-Nitrosodimethylamine	62-75-9	74.08	151 °C	Volatile
N-Nitrosodiethylamine	55-18-5	102.14	172 °C	Volatile
N-Nitrosodipropylamine	621-64-7	130.19	206 °C	Volatile
N-Nitrosodiethanolamine	1116-54-7	134.13	114 °C	Volatile
N-Nitrosodibutylamine	924-16-3	158.24	235 °C	Volatile
N-Nitrosodiphenylamine	86-30-6	198.22	101 °C	Volatile
N-Nitrosodicyclohexylamine	947-92-2	210.32	350 °C	Semivolatile

Why volatility has to be addressed

01 Lower effective concentration

Partial evaporation reduces the dose to which bacteria are exposed.

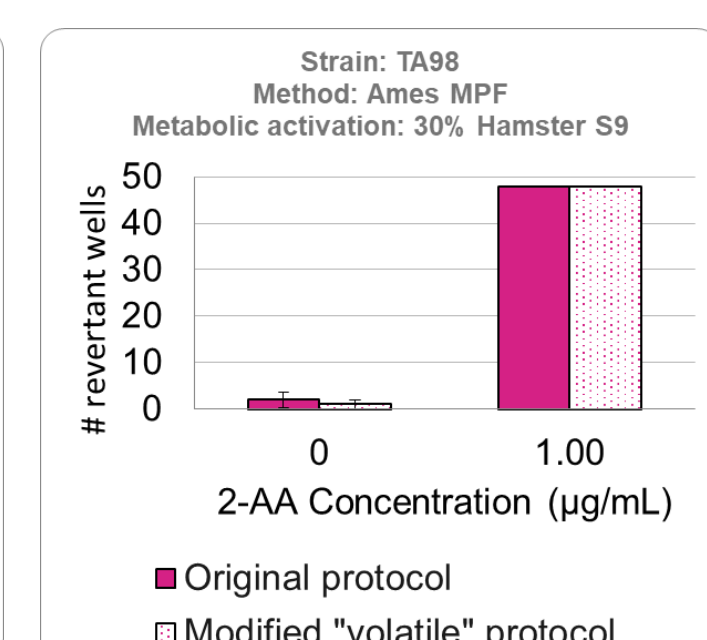
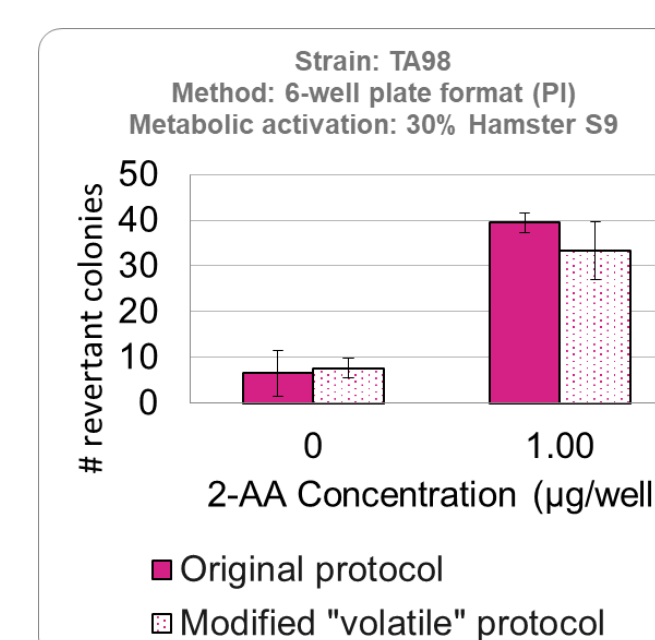
02 Cross-contamination

Neighbouring wells and concurrent controls can be affected → invalid results.

03 Occupational hazard

The operator may be exposed to volatile, toxic substances.

The solution



Both the 6-well format and Ames MPF run under the adjusted protocol with unchanged negative and positive controls.

Conclusion

Miniaturized Ames assays reliably assess nitrosamine mutagenicity and, with protocol adjustments, accommodate both volatile and non-volatile substances. Concordance with the Petri dish-based Ames test was excellent: 7/8 positive nitrosamines were positive in MicroAmes6, and 6/8 in Ames MPF (remaining two equivocal). Between the two miniaturized formats, results matched exactly for 8/12 substances, with discordance only for N-Nitrosodicyclohexylamine and Streptozotocin. Miniaturized Ames assays thus provide a resource- and environment-friendly alternative to Petri dish testing, with strong predictive power for nitrosamines and other genotoxic impurities, plus 3R benefits: less S9, less compound, and minimal plastic waste.

The Ames MPF and MicroAmes6 are proprietary miniaturised Ames assays developed by Xenometrix AG. Check out our homepage xenometrix.ch for further information, and follow us on social media:

