

# Genotoxicity Evaluation of Nitrosamines Using Miniaturized Ames Assays and In Vitro Micronucleus Test

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## Introduction

Detecting genotoxic impurities in pharmaceuticals, packaging materials, and other consumer and industrial products is essential for public health protection, which requires robust and reliable safety assessment methods. International concerns about nitrosamines have led to collaborative efforts to improve testing strategies for these compounds. When evaluating the mutagenic potential of a nitrosamine through in vitro testing, regulatory authorities recommend using the Enhanced Ames Test – EAT [1] [2]. A negative result from a properly conducted enhanced Ames assay can justify setting a higher Acceptable Intake (AI) limit for that nitrosamine.

## Highlights

- Miniaturized genotoxicity testing closes critical data gaps in early development
- Advanced FISH probes deliver a direct result on mode of action
- Enhanced Ames Test for Nitrosamines are relevant for industry, public health
- Miniaturized methods are resource-efficient and minimize contaminated waste

## Methods

In this study, we compared two miniaturized versions of the Ames assay: the agar-based 6-well Ames test (MicroAmes6), and the liquid microplate fluctuation assay (Ames MPF). In compliance with Enhanced Ames Test (EAT) [1] [2], and HESI recommendations [3], the following Ames tester strains were applied: TA100, TA1535, and E. coli uvrA[pKM101] as well as the 30% hamster liver S9. Cytokinesis-block in vitro micronucleus assay was conducted to further investigate the Nitrosamine test samples. The simultaneous application of telomere and centromere-specific FISH probes provided information on the mode of action of the test compound, such as clastogenicity and aneugenicity.

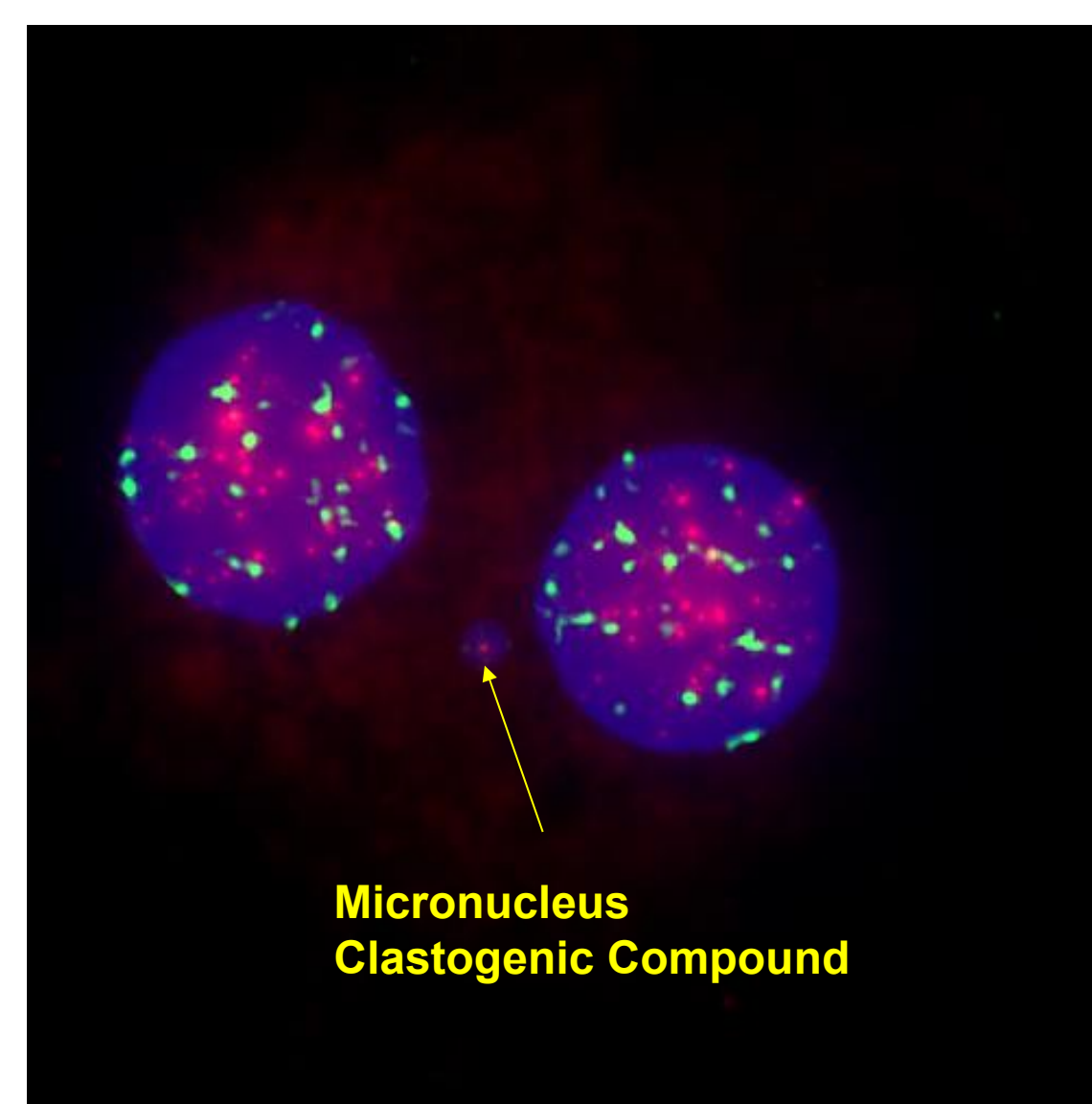
## Results

### In Vitro Micronucleus Assay with Telomere and Centromere-Specific FISH Probes – Mode Of Action Insights



Treatment (µg/mL)	Micronuclei with Telomere	Micronuclei with Telomere and Centromere	Ratio Clastogen	Ratio Aneugen
WATER	14	1	14.0	0.1
2000	9	1	9.0	0.1
CP 6.7	23	2	11.5	0.1
CP 10	26	3	8.7	0.1

NDMA (N-Nitrosodimethylamine) exposed to human whole blood in 3 + 21 hours treatment with hamster liver S9 metabolic activation. Limited number of concentrations were analyzable due to cytotoxic effects of NDMA in a preliminary dose-range finding experiment. Water was applied as negative control, Cyclophosphamide (CP) was applied as positive control.



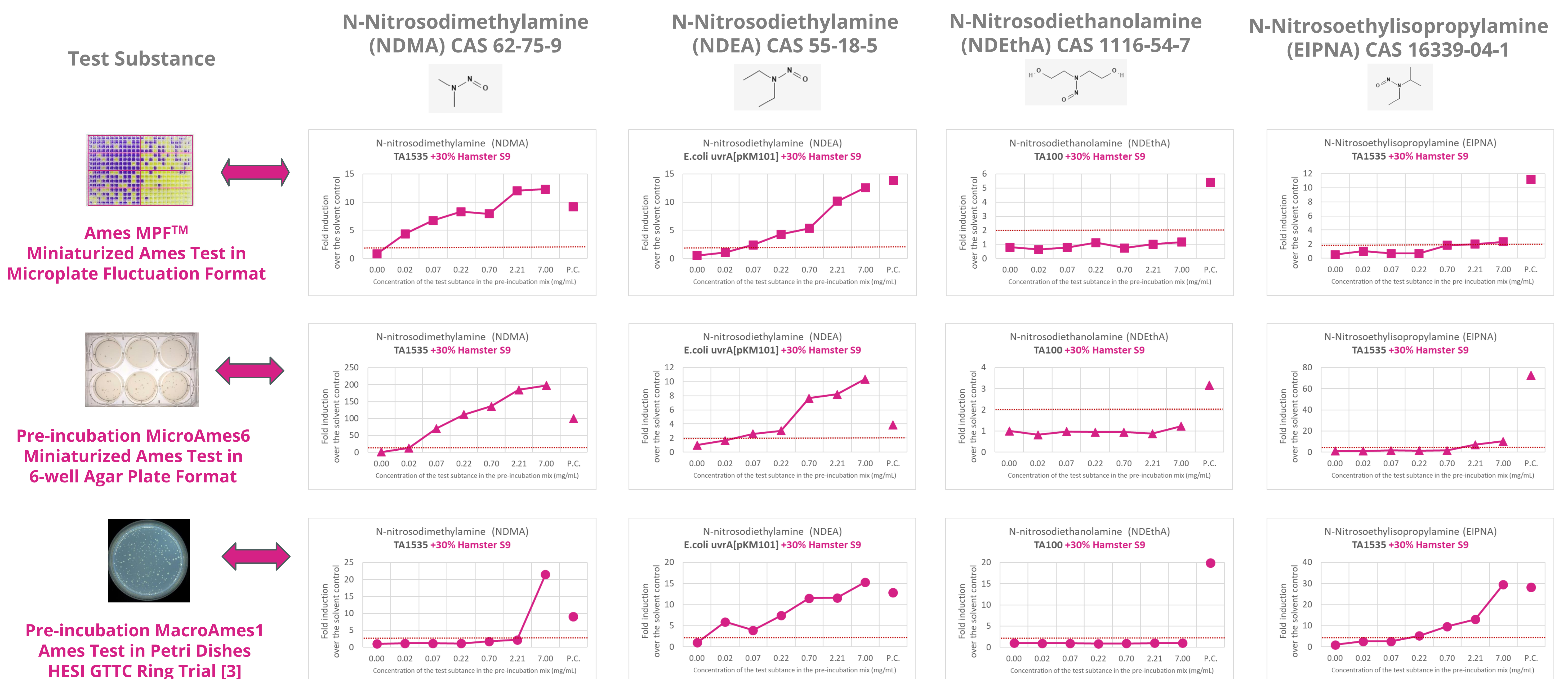
Blue = DNA  
Red = Telomere Probe  
Green = Centromere Probe



Treatment (µg/mL)	Total Cells	Cells with Micronuclei	Micronuclei with Telomere	Micronuclei with Telomere and Centromere	Ratio Clastogen	Ratio Aneugen
DMSO	5779	16	12	2	6.0	0.2
4000	3815	21	17	3	5.7	0.2
4500	3925	20	16	3	5.3	0.2
5000	3926	22	19	2	9.5	0.1
CP 6.7	3887	46	48	1	48.0	0
CP 10	3910	63	57	8	7.1	0.1

NCP (1-Cyclopentyl-4-nitrosopiperazine) exposed to human whole blood in 3-hour treatment with hamster liver S9 metabolic activation. DMSO was applied as negative control, Cyclophosphamide (CP) was applied as positive control.

### Miniaturized Ames Test Results under EAT Conditions - Comparison with Petri Dish-based Ames Test Data in the Literature



## Conclusion

This study highlights how miniaturized versions of two well-established genetic toxicity tests — the Ames assay and the in vitro micronucleus test — can help fill critical data or information gaps in the safety assessment of nitrosamines. The miniaturized Ames assays - as scaled-down alternatives to the traditional Petri dish-based assay - retain scientific rigor while requiring few resources (S9, plastic ware, waste), making them a practical step for early discovery projects, when the amount of available test compound is limited and fast decision-making is required. The in vitro micronucleus assay with the telomere- and centromere-specific FISH Probes facilitates the better understanding of the mode of action of Nitrosamine genotoxicity. Moreover, it provides a fast (3 hours) and robust basis for the differentiation between aneugens and clastogens. The data generated through this work will support more informed safety evaluations of nitrosamines and related genotoxic impurities, with broad relevance across the pharmaceutical and chemical industries. Ultimately, this research contributes to stronger public health safeguards, more efficient regulatory decision-making, and a shift toward greener, more sustainable laboratory practices.

References:  
[1] Food & Drug Administration. (2023, August). Control of Nitrosamine Impurities in Human Drugs. Guidance for Industry  
[2] European Medicines Agency. (2023, July). Questions and answers for marketing authorization holders/applicants on the CHMP Opinion for the Article 5CB of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products  
[3] Bercu J, Trejo-Martin A, Chen C, Schuler M, Cheung J, Cheairs T, Lynch AM, Thomas D, Czich A, Atrakchi A, McGovern TJ, Heflich RH, Vespa A, Frootchi R, Yang Y, Gandhi RD, Elloway J, Ziegler V, Hellmann A, Schaefer M, Tennant RE, Westerink W, Hoffmanns R, Jolly R, Noteboom J, Gollapudi P, Sobol Z, McCreight KK, Christensen JS, Simon S, Dieckhoff J, Zeller A, Marchand C, Waseo K, Bishop ME, Leavitt P, Hargreaves V, Glick C, Liao Y, Elspuru R, Puglisi R. HESI GTTC ring trial: Concordance between Ames and rodent carcinogenicity outcomes for N-nitrosamines (NAs) with rat and hamster metabolic conditions. Regul Toxicol Pharmacol. 2025 Sep;161:105835. doi: 10.1016/j.yrtph.2025.105835. Epub 2025 Apr 29. PMID: 40311791

The Ames MPF and MicroAmes6 are proprietary miniaturised Ames assay kits developed and manufactured by Xenometrix. The in vitro micronucleus assay with FISH probes is available as a service under GLP or non-GLP conditions from GenEvolution. The Rapid Micronucleus MoA Telomere Centromere Kit is a ready to use kit available from Xenometrix. Check out the homepages [www.xenometrix.ch](http://www.xenometrix.ch) and [www.genevolution.fr](http://www.genevolution.fr) for further information, and follow us on social media.