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An Assessment of Four Historically Discordant Nitrosamines in the Ames Test

Introduction

The Ames Test is a quick turn around assay that is able to identify gene mutations in the form of base pair substitutions and frameshift mutations using genetically modified (at histidine or tryptophan loci) strains of *Salmonella typhimurium* and *Escherichia coli*.

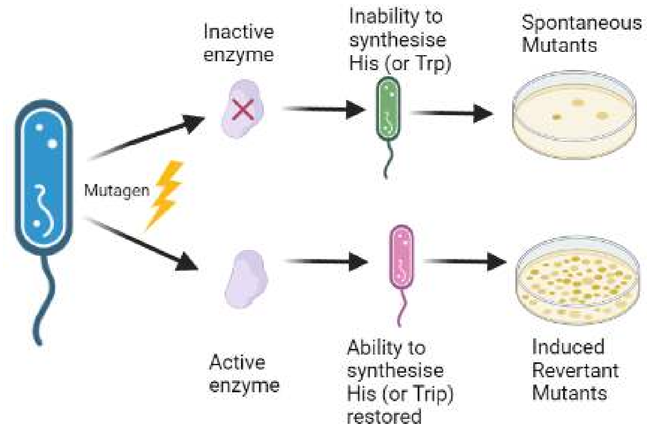

Fig 1. *Formation of spontaneous & induced mutant colonies within the Ames Assay.*

Nitrosamines (NAs) are a class of chemical recently identified as contamination impurities in some pharmaceutical products. This has raised regulatory concern due to their high potency in pre-clinical assays for mutagenesis and rodent carcinogenesis. Historically the dis-concordant results for several nitrosamines vis a vis the Ames Test and rodent carcinogenicity bioassay, has questioned the reliability of a negative Ames Test results to support regulatory decisions regarding their control as impurities in drug product.

Most “dis-concordant NAs” were tested prior to the introduction of OECD 471 guidelines for the bacterial reverse mutation assay, which has prompted a re-evaluation by GSK of several exemplar NAs using an enhanced Ames Test study design for NAs that also reflects the current standards (see below).

Methods

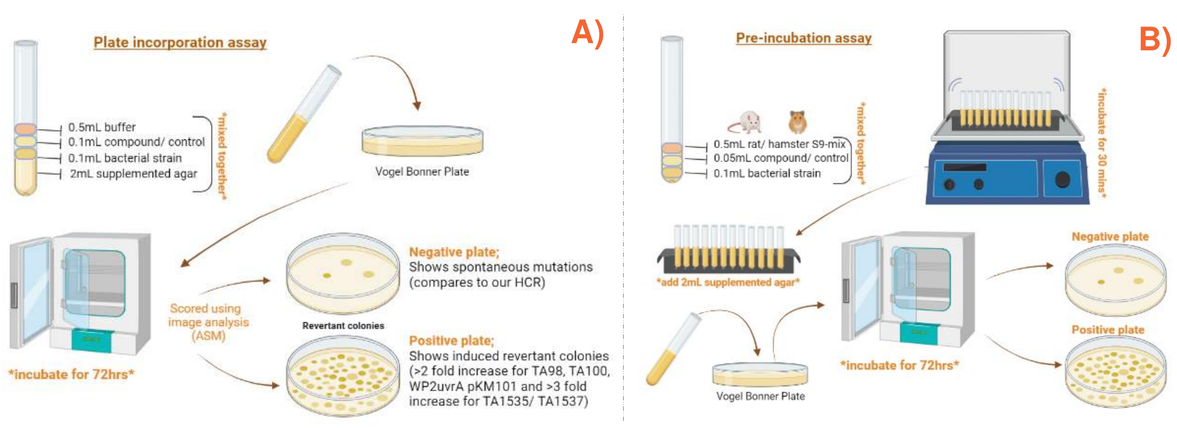

Fig 2. *Formation of spontaneous & induced mutant colonies within the Ames Assay via A) the plate incorporation method and B) the pre-incubation method.*

These methods were conducted in the spirit of GLP following OECD 471 and ICH S2(R1) guidelines. These tests parameters (see accompanying poster by Williams et al) were selected to enhance the sensitivity of the assay for the testing of NAs (including a 30 minute pre-incubation step for treatments with the presence of S9-mix and using a plate incorporation method in the absence of S9-mix).

Nitrosamines under assessment		
Methylethylnitrosamine	CAS 10595-95-6	●
2-Methoxy-N-(2-methoxyethyl)-N-nitrosoethanamine	CAS 67856-65-9	●
N-Methyl-N-nitroso-2-propanamine	CAS 30533-08-5	●
Methyl(4-fluorophenyl)nitrosamine	CAS 937-25-7	●

Aims/ objectives

- Test each NA in a plate incorporation assay in the absence of S9-mix, pre-incubation in the presence of rat S9-mix, and pre-incubation in the presence of hamster S9-mix in each tester strain.
- Evaluate whether the four NAs must still be deemed dis-concordant once they have undergone their OECD-compliant Ames Tests.
  - Highlight any results flagging positive for mutagenicity in the Ames Test and assess their potency.

Results

The pre-incubation Ames Test results:

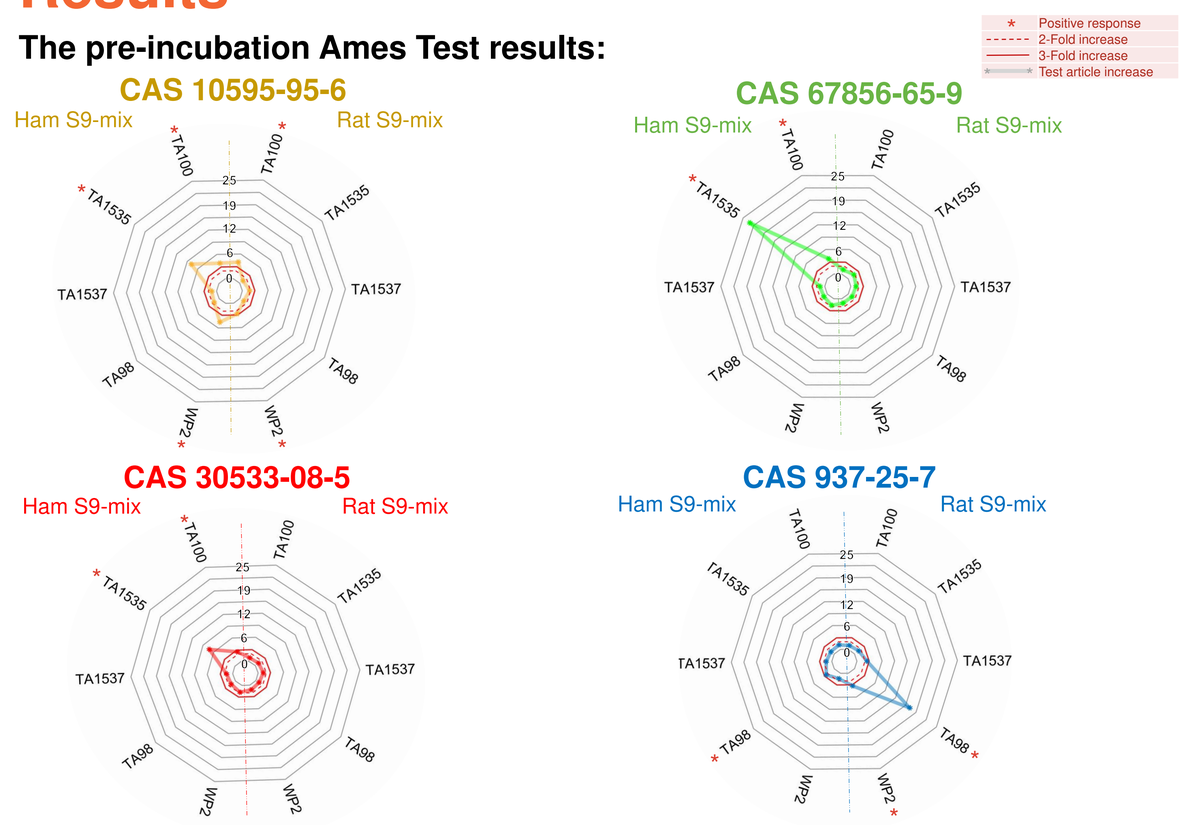

Fig 3. *Pre-incubation radar plots of mutagenicity for CAS 937-25-7, CAS 67856-65-9, CAS 30533-08-5, & CAS 10595-95-6. These plots show the highest revertant count ratio (NA concentration compared with solvent control) for each bacterial strain.*

The criteria for a positive response in the Ames test is a >2 fold increase in revertant colonies for strains TA98, TA100 and WP2uvrA(pKM101) and a >3 fold increase for strains TA1535 and TA1537.

Test article related increases in revertant colonies for CAS 10595-95-6 (≥ 2/3 fold concurrent control) were observed in strains TA100 and WP2 uvrA (pKM101) in the presence of rat-S9-mix (4.2 and 2.7-fold increases, respectively), and in TA100, TA1535, and WP2 uvrA (pKM101) in the presence of hamster S9-mix (4.0, 8.2 and 4.8-fold increases, respectively), therefore, indicating a positive result.

Test article related increases in revertant colonies for CAS 67856-65-9 (≥ 2/3 fold concurrent control) were observed in strains TA100 and TA1535 in the presence of hamster S9-mix (3.9 and 23.0-fold increases, respectively), therefore, indicating a positive result.

Test article related increases in revertant colonies for CAS 30533-08-5 (≥ 2/3 fold concurrent control) were observed in strains TA100 and TA1535 in the presence of hamster S9-mix (2.6 and 7.5-fold increases, respectively), therefore, indicating a positive result.

Test article related increases in revertant colonies for CAS 937-25-7 (≥ 2/3 fold concurrent control) were observed in strains TA98 and WP2 uvrA (pKM101) in the presence of rat-S9-mix (16.8 and 3.3-fold increases, respectively) and in TA98 in the presence of hamster S9-mix (2.5-fold increase), therefore, indicating a positive result.

The plate incorporation Ames Test results:

No test article related increases in revertant colonies (≥ 2/3 fold concurrent control) were observed in the plate incorporation test in the absence of S9-mix for CAS 10595-95-6, CAS 67856-65-9, CAS 30533-08-5, or CAS 937-25-7 and therefore expressed negative results for mutagenicity in this treatment arm. This also confirms the NA need to be metabolically activated in order to produce a positive response in the Ames test.

Conclusions

The strains TA100, TA1535 and WP2uvrA(pKM101) are the most sensitive for showing a positive response in the Ames test for NAs. However, in the pre-incubation tests for CAS 937-25-7 (in both rat/ hamster S9-mix) a positive response was also seen in TA98. This could suggest further impurities are driving this outcome.

The results of these four compounds confirm that an OECD complaint Ames Test can detect the mutagenic activity of historically discordant NAs. Therefore, it is concluded that these four NA compounds should no longer be considered as an Ames Test discordant.

Acknowledgements

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