Is the bacterial reverse mutation assay an accurate predictor for N-nitrosamine carcinogenicity?

Abstract 274

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OBJECTIVES

- To investigate if the Ames test is sufficiently predictive of *in vivo* carcinogenicity for N-nitrosamine compounds.
- Analyse currently available data for protocol variations:
  - Strain (all versus OECD 471-compliant)
  - S9 metabolic activation (rat versus hamster)
  - Protocol (preincubation versus plate incorporation)
  - Solvent (DMSO versus water)

APPROACH

- Studies related to N-nitrosamines were extracted from the Lhasa Limited Vitic 2020.1 Database and subsequently categorised according to the conditions used.
- The predictive performance of the Ames test for carcinogenic activity was analysed.
- The impact of varying the study conditions on the predictive performance was assessed.

MAIN RESULTS

- The Ames data correctly predicted the carcinogenic activity of 85% of N-nitrosamines (117 of 138 compounds).
- S9 type, solvent and protocol variations had little effect on the predictive performance of the Ames test overall.
- The preincubation protocol and non-DMSO solvent may improve sensitivity for short chain aliphatic nitrosamines.
- Hamster S9 may lead to improved sensitivity versus rat S9 at lower compound concentrations.

IMPACT

- Ames test conducted under OECD guidelines is highly sensitive for predicting carcinogenicity of N-nitrosamines.
- Protocol variations that may lead to improved sensitivity.
- Optimal conditions for testing of N-nitrosamines may improve both the accuracy and confidence in the ability of the Ames test to identify potential carcinogens
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OBJECTIVES

- N-nitrosamine (NA) impurities have recently been discovered in several marketed pharmaceuticals, leading to a requirement for further investigation into their mutagenic and carcinogenic activity.
- Recent regulatory requirements necessitate marketing authorisation holders for human medicines, containing chemically synthesised active substances, to review their products for the possible presence of genotoxic nitrosamines and test extensively where there is a risk.
- The OECD-471 bacterial reverse mutation (Ames) assay is the most widely used primary screen to assess drug impurities for potential mutagenic risk to patients and is a first screen for carcinogenic potential.
- Previous literature reports indicated that the Ames test might not be sensitive enough to detect the mutagenic potential of NA compounds in order to accurately predict a risk of carcinogenicity.
- Firstly, we aim to investigate if the Ames test is sufficiently predictive of *in vivo* carcinogenicity for NA compounds.
- Secondly, we plan to analyse currently available data to determine the effect of the following protocol variations:
  - Strain (all versus OECD 471-compliant)
  - S9 metabolic activation (rat versus hamster)
  - Protocol (preincubation versus plate incorporation)
  - Solvent (DMSO versus water)

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APPROACH

- Studies related to NA compounds, with the exclusion of nitrosocarbamates, nitrosoureas and nitrosoamides, were extracted from the Lhasa Vitic 2020.1 Database.
- Data related to the Ames and rodent carcinogenicity studies were subsequently filtered and analysed.
- Overall mutagenicity and carcinogenicity calls were assigned for each compound to enable a comparison of mutagenic and carcinogenic activity.
- In order to explore the impact of variations in the Ames study conditions on its predictivity for carcinogenesis, the Ames data was separated into subgroups according to the below criteria:
    - All Ames assay data.
    - Using OECD compliant bacterial strains only.
    - Using OECD strains with rat S9 as metabolic activation.
    - Using OECD strains with hamster S9 as metabolic activation.
    - Using OECD strains according to the plate incorporation protocol.
    - Using OECD strains according to the preincubation protocol.
    - Using OECD strains under plate incorporation protocol with rat S9 metabolic activation.
    - Using OECD strains under preincubation protocol with rat S9 metabolic activation
    - Using OECD strains with dimethylsulphoxide (DMSO) as solvent.
    - Using OECD strains with water as solvent.

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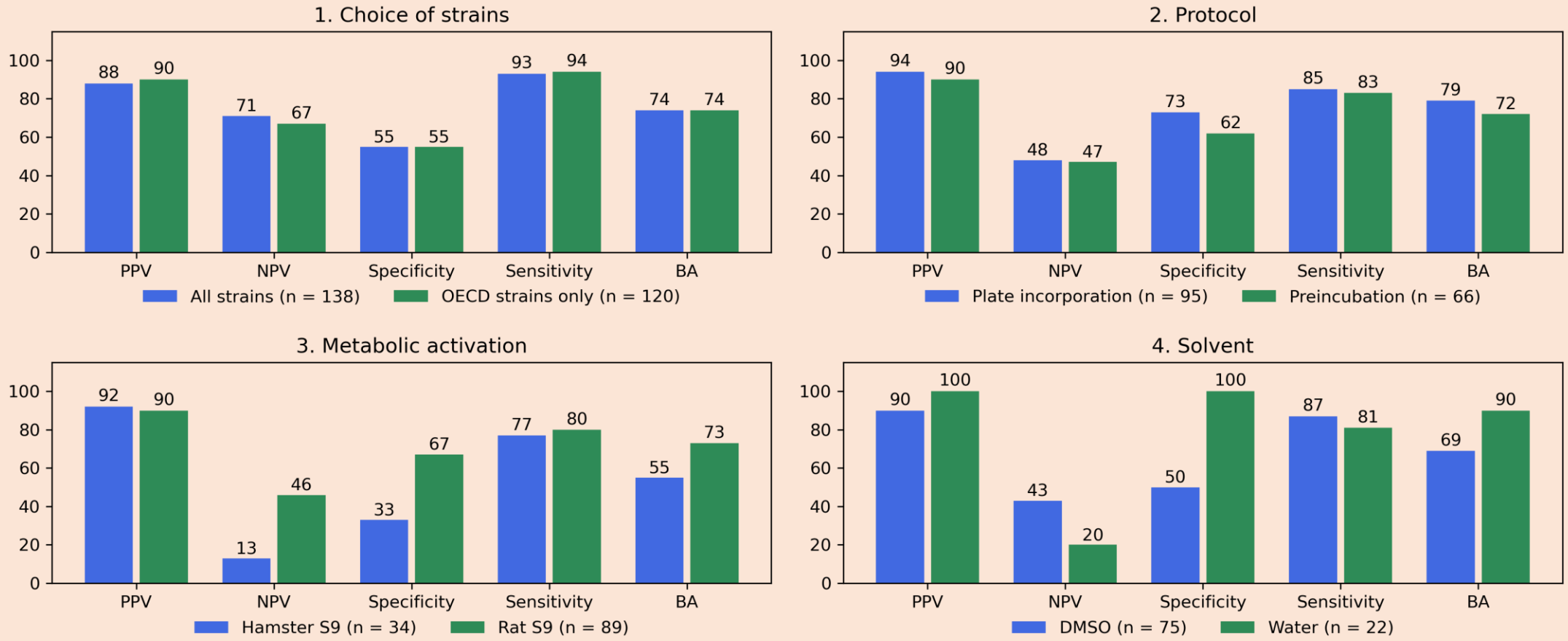
MAIN RESULTS – Performance metrics of the Ames test vs Carcinogenicity data



1. The Ames test conducted under current OECD guidelines is highly sensitive for predicting carcinogenicity of N-nitrosamine compounds.
2. Protocol made little difference for medium-large N-nitrosamines, but preincubation may improve sensitivity for short chain aliphatic N-nitrosamines.
3. Hamster versus rat S9 made little difference to sensitivity considering the OECD-recommended strains. However, where compounds were tested up to relatively low concentrations in the Ames test (1000-2000 µg/plate), hamster S9 gave a positive response for positive carcinogens in sensitive strains for N-nitrosamines (TA1535 and TA100) more frequently than rat S9.
4. Solvent made little difference, except for certain simple alkylnitrosamines (e.g., NDMA, NDEA) which demonstrate reduced mutagenic activity with DMSO.

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IMPACT/SIGNIFICANCE

- We have demonstrated that the Ames test conducted under current OECD guidelines is highly sensitive for predicting carcinogenicity of NA compounds.
- We have presented how variations to the OECD 471-compliant Ames test, including type of metabolic activation, solvent type and pre-incubation/plate incorporation methods, may impact the predictive performance for carcinogenicity.
- Larger N-nitrosamines are less sensitive to variation in testing procedure than small aliphatic dialkyl compounds
- Further understanding of optimal conditions for testing of N-nitrosamines may improve both the accuracy and confidence in the ability of the Ames test to identify potential carcinogens.