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Comparison of sensitivity between the spot test and the miniscreen assay

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Keywords: Ames-simple method, spot test, miniscreen assay

The spot test (ST) and the miniscreen assay (MA) are filter paper diffusion and down-sized versions of the standard Ames assay, respectively. ST requires <1/25 of a given compound than the standard Ames assay, whereas MA requires <1/5 of the compound. In this study, we conducted ST and MA with “in-house” Ames-positive compounds, and compared the sensitivity between ST and MA using the 2 bacterial tester strains, TA100 and TA98. For ST, a filter paper soaked with the compound solution was placed on an 8.5cm agar-plate with PBS/S9mix and the bacterial solution. For MA, the compound solution, PBS/S9mix, the bacterial solution and soft agar were added to a 12-well agar-plate.

ST was able to dose-dependently detect AF2 and 2AA, typical Ames-positive compounds, but detected few “in-house” Ames-positive compounds that increased >1000 revertant colonies. In contrast, MA was able to detect the “in-house” Ames-positive compounds that increased the revertant colonies >3-fold in TA100, >5-fold in TA98. The Ames-negative and MA-negative results were consistent with similar strains independent of the S9 mix.

Consequently, we confirm that MA exhibits higher sensitivity than ST, and can be useful in detecting only the strongly Ames-positive compounds without false-positive results.

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Mutation spectra of N-acetoxy-3-aminobenzanthrone, a derivative from the environmental pollutant 3-nitrobenzanthrone, in human cells


3-Nitrobenzanthrone (NBA) is a suspected human carcinogen identified in diesel exhaust and airborne particle. We synthesized its possible reactive intermediate, N-acetoxy-3-aminobenzanthrone (N-Aco-ABA), to yield the DNA adducts of NBA. To evaluate the mutagenic specificity of NBA in human cells, N-Aco-ABA was reacted with plasmid containing the supF reporter gene, then the plasmid was transfected into nucleotide excision repair (NER)-proficient and NER-deficient fibroblast cells [WI38-VA13 and XP2OS(SV), respectively]. In both cells, the number of replicated plasmid decreased, and the mutation frequency augmented with increasing concentration of N-Aco-ABA. Base sequence analysis of plasmids with mutations in the supF gene revealed the majority of the mutations were base substitutions (89 and 94%). More than 80% of the mutant plasmids had a single base substitution in XP2OS(SV), whereas 49% of mutant plasmids had multiple base substitutions and 46% had single base substitution in WI38-VA13. Of the base substitutions, the most frequent mutation was G:C to T:A transversion (42 and 38%) in both cells, followed by G:C to A:T transition in WI38-VA13 (28%), A:T to G:C transversion in XP2OS(SV) (22%). The mutations were distributed not randomly but located at several hot spots, and almost all hot spots were at the sites of G:C base pairs.

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