Author's response to reviews

Title: Characterisation of an aerosol exposure system to evaluate the genotoxicity of whole mainstream cigarette smoke using the in vitro gammaH2AX assay by High Content Screening

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Author's response to reviews: see over
The authors have been responsive to the reviewers' comments; however, a statistical analysis cannot be performed with only two samples. At least three conditions/sample need to be included for statistical analysis. This manuscript requires further revisions.

Further clarification was requested as it was not clear to the authors what the editor meant.

“The authors can use a t-test to perform statistical analysis, but at least 3 replicates/sample is needed to be valid.”

We agree with the editor on the point made regarding the number of samples to be used on a statistical analysis. We have carefully reviewed our previous answer and we believe there has been a misinterpretation. On the second paragraph of comment number 2 from the previous response to Editor we wrote:

“Nevertheless, We have performed a 2 sample t-test statistical analysis to evaluate the concern over reduced assay response. When etoposide data from both set of experiments were analysed, statistical differences were observed (p<0.001). We then performed the same statistical analysis in untreated controls and air-treated controls to confirm if a reduction in response was also observed at the baseline level. The Untreated controls produced a p=0.203 while air-treated controls produce a p=0.855. These results confirm that the assay performance was not reduced while testing M4A samples. Positive controls such as Etoposide produce an exaggerated response to ensure responsiveness of the \textit{in vitro} system and this could often produce more variability in the signal intensity.”

We apologise for the confusion created. We did not mean to say that we used two samples to perform the analysis but that the analysis was a “2 sample t-test” where two groups of data means are compared. We have clarified in our materials and methods that the experiments were replicated at least 3 times with 4 repeats per dilution per experiment for the whole mainstream cigarette smoke evaluation.