

2<sup>nd</sup> International Conference on

# Food Safety and Regulatory Measures

June 06-08, 2016 London, UK

## Genotoxicity evaluation of cigarette smoke condensates using the *in vitro* $\gamma$ H2AX assay by flow cytometry

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The measurement of serine139-phosphorylated histone H2AX ( $\gamma$ H2AX) provides a biomarker of DNA double-strand breaks (DSBs) and may identify potential genotoxic activity. In order to evaluate genotoxicity of cigarette smoke condensates, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 2-amino-9H-pyrido[2,3-b] indole (A $\alpha$ C) were selected to perform a  $\gamma$ H2AX assay by flow cytometry for rapid detection of  $\gamma$ H2AX focus. Chinese hamster ovary (CHO) cells were exposed to NNK for 24 h in concentrations of 0, 25, 50, 100, 200, 400  $\mu$ g/mL. CHO cells were treated with A $\alpha$ C for 24 h in concentrations of 0, 2.5, 5, 10, 20, 40  $\mu$ g/mL. Cell viability was measured by MTT assay. Flow cytometry was employed to detect  $\gamma$ H2AX focus, which detects DNA double strand breaks in a high-throughput mode. Then, cigarettes of 12 different brands were selected. Series of concentrations of 0, 9.375, 18.75, 37.5, 75, 150  $\mu$ g/mL were used to evaluate their genotoxicity via detect the  $\gamma$ H2AX focus by flow assay. The results showed that 1) There was a significant increase in  $\gamma$ H2AX frequency in both NNK and A $\alpha$ C in a concentration-dependent manner. A positive response was observed after 24 h treatment at concentrations above 25  $\mu$ g/mL of NNK or 10  $\mu$ g/mL of A $\alpha$ C, respectively. 2) All of 12 cigarette smoke condensates produced positive genotoxic response in a concentration-dependent manner. It indicated that the *in vitro*  $\gamma$ H2AX assay by flow cytometry could be used as a pre-screening tool to assess the genotoxicity effect of cigarette smoke condensates.

### Biography

Xiang Li has completed his PhD at the age of 29 years from Beijing Institute of Basic Medical Sciences. He is associate professor in Zhengzhou Tobacco Research Institute of CNTC and his research field is toxicology of tobacco smoke and risk assessment of tobacco products. He has published more than 20 papers in reputed journals.

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