

Toxicology Mechanisms and Methods

ISSN: 1537-6516 (Print) 1537-6524 (Online) Journal homepage: http://www.tandfonline.com/loi/itxm20

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To cite this article: Sen Zhang, Huan Chen, An Wang, Yong Liu, Hongwei Hou & Qingyuan Hu (2018): Genotoxicity evaluation of carbon monoxide and 1, 3-butadiene using a new joint technology — the in vitro γH2AX HCS assay combined with air-liquid interface system, Toxicology Mechanisms and Methods, DOI: 10.1080/15376516.2018.1477897

To link to this article: https://doi.org/10.1080/15376516.2018.1477897

Accepted author version posted online: 21 May 2018.

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Genotoxicity evaluation of carbon monoxide and 1, 3-butadiene using a new joint technology — the *in vitro* γH2AX HCS assay combined with air-liquid interface system

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Abstract: To investigate the genotoxicity of gaseous toxicants CO and 1,3-butadiene in vitro, a novel combination technology-the *in vitro* γH2AX high content screening assay combined with air-liquid interface system was established. The results showed that this new technology was available and effective. Based on the joint technology, genotoxicity of CO and 1,3-butadiene was evaluated further in this study. The results showed that treatment concentrations (0, 20%,40%, 80% and 100%, v/v) and exposure time (15, 30, 45, 60 and 90 min) of CO both had no statistically significant effects on the induction of γH2AX ($p>0.05$). However, 1,3-butadiene can induce significant γH2AX ($p<0.01$) in A549 cells in a dose/time-dependent manner both in the absence and presence of rat liver S9. When the concentrations of 1,3-butadiene were more than 80 %, a higher γH2AX level could be induced than the 1.5-fold of vehicle controls after 1 h of treatment. Overall, this new technology can be used a complementary tool to evaluate the genotoxicity of airborne toxicants in vitro based on the *in vitro* γH2AX high content screening assay combined with air-liquid interface system. Based on the joint technology, CO was not genotoxic in A549 cells, while 1,3-butadiene showed significant genotoxicity in the dose/time-dependency on the induction of γH2AX.

Keywords: γ H2AX; Genotoxicity; High content screening; Carbon monoxide; 1,3-butadiene; Air-liquid interface system

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supplemented with 10% foetal bovine serum (Gibco, Grand Island, USA). Cells were seeded into 25-cm^2 culture flask and maintained at 37 ºC and 5% CO^2 in a humidified incubator. Before exposure, phosphate buffer saline (PBS) was added in the transwell (bottom, 2 mL; top, 0.5 mL). Then, the transwell was incubated at 37 ºC and 5% CO^2 for 1-2 h as an initial equilibrium time to improve cell attachment. PBS buffer was then removed from the top and bottom of the inserts, and replaced with fresh culture media (bottom, 2 mL; top, 0.5 mL) containing 10 mmoL/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (Solarbio, Beijing, China). Cell in logarithmic phase were enzymatically removed from 25-cm^2 culture flask using 0.25% Trypsin/EDTA (Gibco, Grand Island, USA), and resuspended in culture medium. Next, the cells were grown on porous polyester membranes with a cell suspension 2×10^5 cells (pore size, 0.4 μm; diameter 24 mm) in transwell-clear inserts (Corning, Corning, USA). Cell cultures were incubated at 37 ºC and 5% CO^2 in a humidified incubator for 24 h.

2.3 CO and BUT exposure in ALIS

After incubating in a humidified incubator for 24 h, the medium was removed from newly confluent cells on the top of the transwell inserts, and both inner and basolateral side of inserts were washed twice with PBS. The transwell inserts were then transferred into the inner chamber of ALIS (as illustrated in Fig. 1B) (Beijing Huironghe, Beijing, China). Cells were exposed to the filtered the mixture gas of CO/BUT and air on the apical side of transwell inserts, while being nourished from their basolateral side. During exposure, the ALIS consisting of three glass medium-containing wells was kept at 37 ºC by circulating water bath. The mixture gas was drawn into exposure wells through upper chambers at a flow rate of 20 mL/min/well. Air was supplied by an air compressor. Air supply and

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