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Effect of cell treatment procedure on *in vitro* genotoxicity assessment

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Chronic exposure to low-dose contaminants is a major public health issue, so it is important to determine their potential hazard and minimize the risk. Up to now, the majority of in vitro toxicological experiments are conducted after an acute 24h treatment that did not represented the human exposure. Recently, new in vitro approaches have been proposed to study chemical toxicological effect over several days in order to be more predictive of a realistic exposure scenario. In this study, we investigated the genotoxic potential of chemicals with the γH2AX and pH3 biomarkers, with different cell treatment procedure. We tested reference compounds (direct or bioactived clastogen, aneugen and apoptotic inducer) in the human liver-derived HepaRP cell line and used different cell treatment duration, with or without a release period, before genotoxicity analysis. Data were analysed with the Benchmark dose approach. We demonstrated that the detection of clastogenic compounds (notably DNA damaging agent) was more sensitive after three days of repeated treatment compared to one or three treatment over 24h. On the opposite, we observed that aneugenic chemicals were more easily detected as genotoxic after a 24h exposure compared to a 3-day repeated treatment. A 3-day release period after the last treatment, decrease substantially the genotoxicity measurement, whatever the chemical tested. In conclusion, in the cell line used, there are some important difference between a one day acute and a three-day repeated treatment protocol, indicating that different cell treatment procedure may permit to differentiate the chemical genotoxic mode of action.

Keywords:
Genotoxic compounds; repeated exposure; release; γH2AX; pH3.