

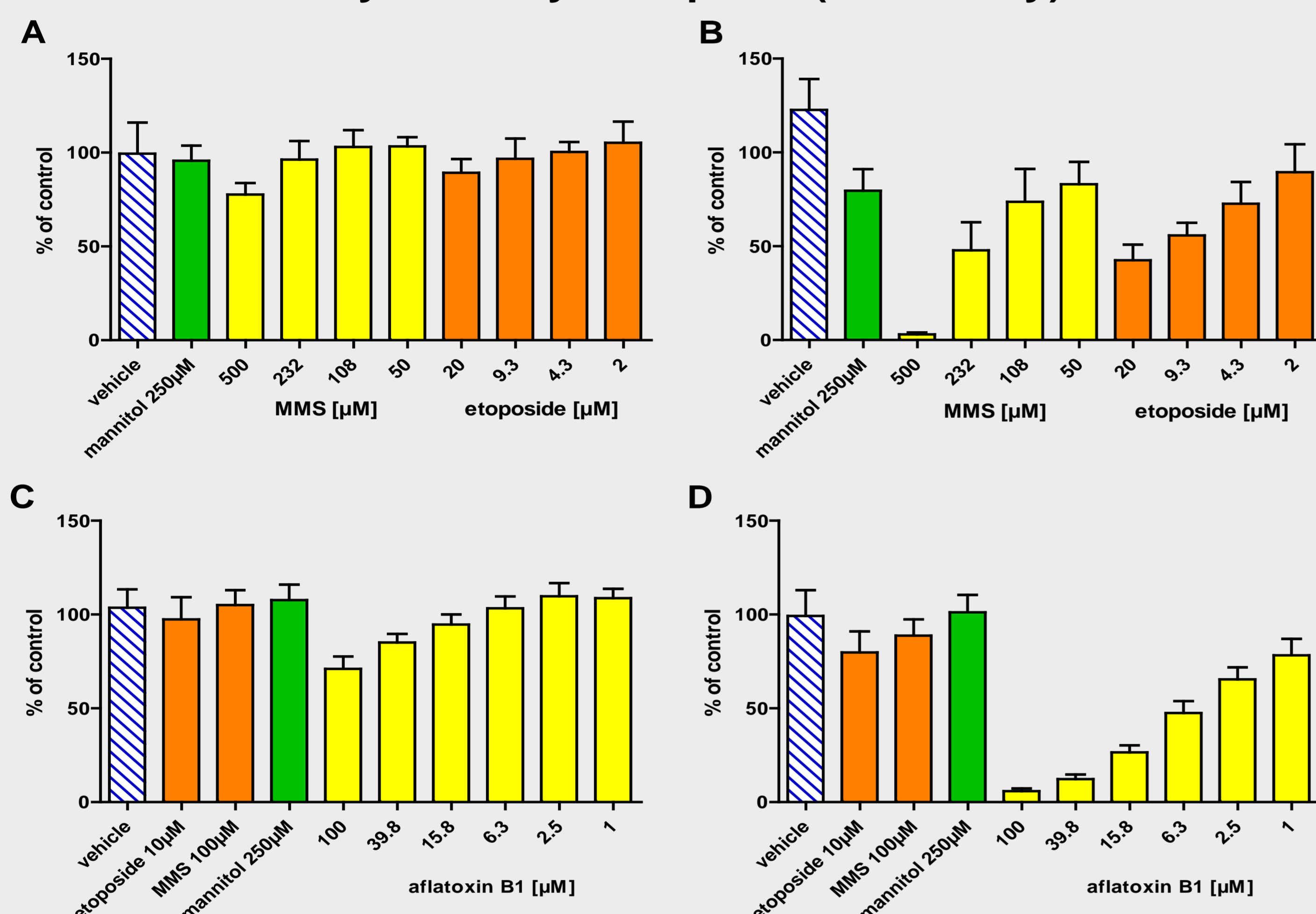
Applicability of HepaRG™ cell line for automated assessment of DNA strand breaks by AUREA gTOXXs

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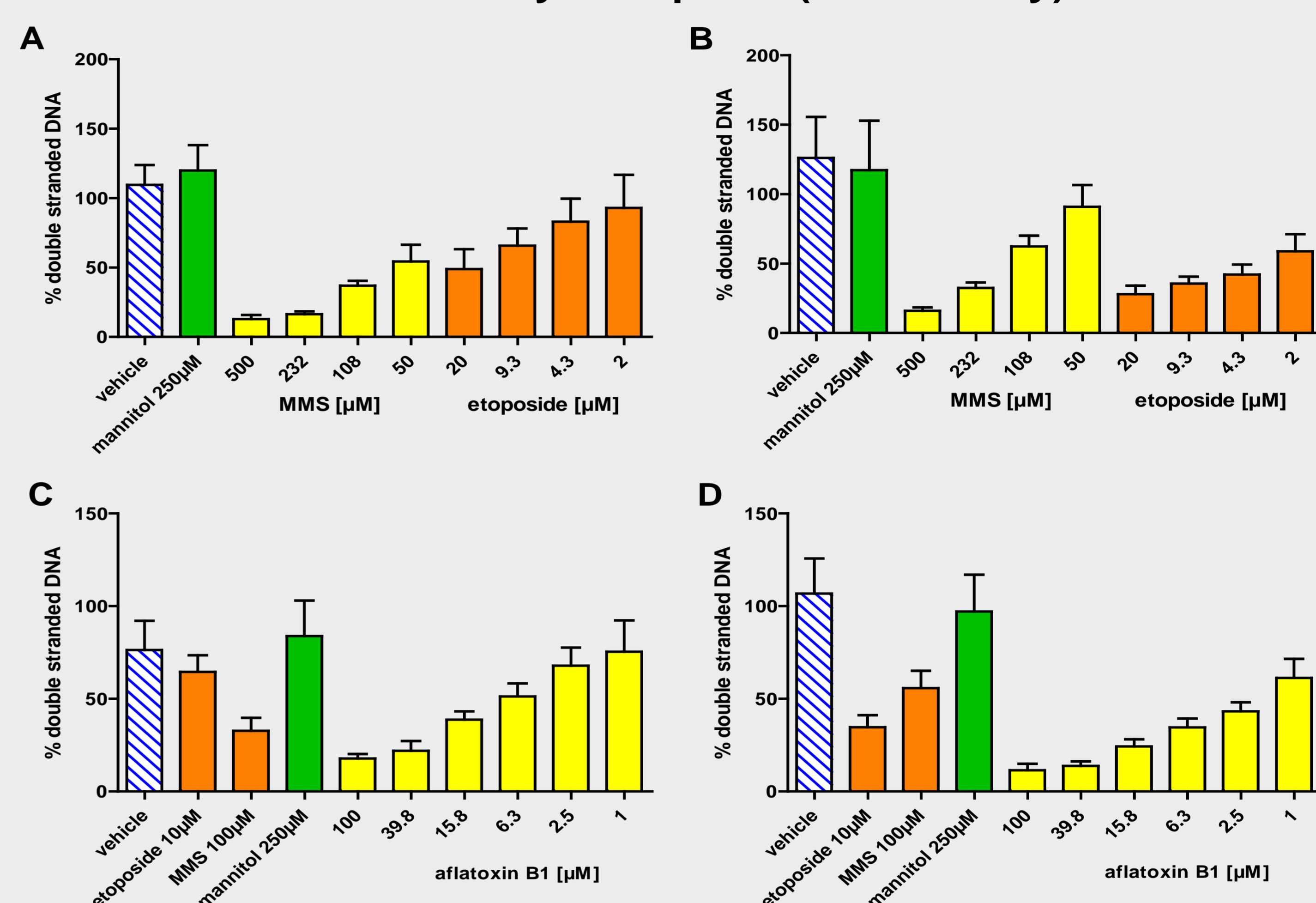
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Cytotoxicity in HepaRG (MTT assay)



Genotoxicity in HepaRG (FADU assay)



HepaRG cells were treated for 3 h (A, C) or 20 h (B, D) with the genotoxic agents, etoposide, methylmethanesulfonate (MMS), or aflatoxin B1 as indicated. Treatments with mannitol (negative) and 0.5% DMSO (vehicle) are included as controls. Data are expressed as a percentage of untreated control samples. Bars represent the mean with standard deviations of at least two separate experiments (n=6).

model for mapping the metabolic response of human hepatocytes to chemicals like aflatoxin which unfolds the toxic effects only upon metabolic activation. The automated gTOXXs solution based on the FADU (Fluorimetric Detection of Alkaline DNA Unwinding) assay provides a sensitive and reliable *in vitro* technique to detect DNA strand breaks. The applicability of the HepaRG™ cell line for gTOXXs based genotoxicity studies of drugs with the need for bio activation by liver cells is demonstrated.

concentrations), however, clear dose-dependent effects on the percentage of DNA strand break (A, C), indicative of their genotoxicity.

- Prolonged treatment with the compounds, etoposide and aflatoxin, generated strong effects with regard to both the cytotoxicity and the genotoxicity at 24 hrs (B,C).
- Interestingly, the percentage of DNA strand breaks caused by MMS treatment is, as yet, decreasing with time of exposure, possibly indicating a DNA repair mechanism in the HepaRG™ cells.

Method

- Cytotoxicity is assessed by the cell viability MTT test.
- DNA strand break is assessed by Aurea gTOXXs: the detection is based on progressive DNA unwinding under specific conditions of alkaline pH, time and temperature and fluorescence labelling of double-stranded DNA (Moreno-Villanueva, Eltze, Dressler et al 2011; Altex, 28, 4/11.)

gTOXXs Analyzer: unique DNA strand break and DNA repair testing platform



- Full automation
- High precision pipetting and temperature control
- Up to three 96-well plates: statistics in one run
- Clean technology: integrated tip washing station

Conclusions

- HepaRG™ cells metabolically affect aflatoxin to produce genotoxic effects
- HepaRG™ cells are applicable for *in vitro* genotoxicity testing
- To better assess the role of metabolic activation in genotoxicity, the HepaRG™ cells could be used before and after differentiation
- It will be interesting to test additional substances to evaluate the HepaRG™ by the gTOXXs

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