Toxicology testing using upcyte® hepatocytes
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INTRODUCTION
Summary and novelty: We have developed a technique which causes primary human hepatocytes to proliferate whilst retaining an adult phenotype. The resulting upcyte® hepatocytes have the capability to proliferate and express sufficient drug metabolizing activities, a combination which makes them unique. So far 5 different donors of upcyte® hepatocytes have been generated (#10, #740, #151, #653, #422).

Cytotoxicity assay: The cytotoxicity of 31 compounds was measured using ATP and LDH content and MTS metabolism in upcyte® hepatocytes from four donors. The cytotoxicity of the majority of compounds was donor-dependent. Donor 653 was generally less susceptible to cytotoxicity than donors 422A, 151 and 10. There was a good intra- and inter-experimental reproducibility and the predictive capacity of the assay was good such that known non-hepatotoxins were clearly negative and compounds that were associated with hepatotoxicity caused cytotoxicity in upcyte® hepatocytes.

Micronucleus assay: We optimized the assay conditions incorporating upcyte® hepatocytes into the micronucleus test. A treatment duration of 96 h was optimal for detecting the genotoxicity of the direct-acting, mitomycin C, and the bioactivated compound, cyclophosphamide, whilst negative and "false" positive compounds were correctly identified as negative. The basal MN rate of upcyte® hepatocytes was affected by pre-culture period and medium components. The% MN in control and genotoxicant-treated upcyte® hepatocytes was similar at different growth stages.

Genotoxicity testing using upcyte® hepatocytes

For cytotoxicity screening, the cells were pre-cultured in 96-well plates at 20 monolayers for 3 days and then treated with test compounds for 4 days. The viability was measured using ATP and LDH content and MTS metabolism in upcyte® hepatocytes from four donors. For some compounds, such as chlorpromazine, the cytotoxicity was similar in upcyte® hepatocytes from different donors (Figure 2A). For other compounds, such as tacrine, the cytotoxicity was donor-dependent. e.g. Donor 653 was generally less susceptible to cytotoxicity than donors 151, 422A and 10 (Figure 2B).

There was a good intra- and inter-experimental reproducibility and the predictive capacity of the assay was good such that known non-hepatotoxins were clearly negative and compounds that were associated with hepatotoxicity caused cytotoxicity in upcyte® hepatocytes. Moreover, there was a good correlation between the MTS IC₅₀ values from our studies with those obtained from the literature for the same compounds in primary human hepatocytes (using MTT), supporting the use of upcyte® hepatocytes as an alternative model to primary cells (Figure 3).

Optimization of treatment and culture conditions for the in vitro MN test
Different treatment (8 to 96 h) and recovery durations (0 to 96 h) were tested to determine the optimal conditions for detecting genotoxicants in upcyte® hepatocytes. Longer treatments resulted in higher formation of MN and lower concentrations of test compound were needed to cause the same extent of MN formation (Figure 4). Figure 5 shows that after a 96 h treatment, the cytotoxicity of etoposide was higher when a subsequent recovery period (without test compound) was included. For this reason, a treatment duration of 96 h, without a recovery period was selected as optimal for all compounds. Under these conditions, upcyte® hepatocytes can be incorporated into the in vitro MN test to detect both directly acting (e.g. mitomycin C, etoposide (Figure 5)) and metabolically activated genotoxicants (e.g. benzo(a)pyrene, cyclophosphamide), whilst true negative and "false" or isomisleading positive compounds were reproducibly and correctly identified as negative (Table 1). The basal MN rate of upcyte® hepatocytes from three other donors was higher than that in Donor 740 (28% compared to 7%, respectively). Therefore, the medium was modified by adding oncostatin M and EGF to decrease inherent MN formation (data not shown). There was a very good reproducibility between experiments with respect to the% MN formed and the cytotoxicity in upcyte® hepatocytes (Figure 6).

The NAMN in control (DMSO) and genotoxicant-treated upcyte® hepatocytes was similar at different growth stages and were within the inter-experimental variation values measured for cells at a population doubling (PD) of 24 (Figure 7).

CONCLUSION
In conclusion, these data support the use of upcyte® hepatocytes in the MN test, especially since these cells combine proliferation with a metabolic capacity – thus negating the need for an exogenous bioactivation system. Our data also show that upcyte® hepatocytes can be used as a suitable alternative to primary human hepatocytes for hepatotoxicity screening-combining predictivity and reproducibility with a substantial cell source.

Table 1: Outcome of the testing of true positive, true negative and false positive chemicals