We conclude that the THLE-CYP cell model enables in vitro investigation of both CYP independent and CYP mediated processes which may contribute to DILI, and also exploration of underlying mechanisms (e.g. bioactivation of clozapine by 2D6). If used in drug discovery, this approach has the potential to aid in rational design and selection of compounds with reduced DILI liability, and could enable early de-selection of drug candidates with significant potential to cause DILI in man.

References
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P26
Evaluation of the relationship between inhibition of the bile salt export pump in vitro and risk of drug-induced liver injury in man
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It has been proposed, that inhibition of hepatobiliary transporters may play an important role in the development of drug-induced liver injury (DILI) (Wagner, 2009; Stieger, 2007). To provide further understanding of the relationship between inhibition of the canalicular transporter bile salt efflux pump (Bsep; Abcb11) and DILI in man, we have assessed inhibition of Bsep activity in vitro by hepatotoxic and non-hepatotoxic marketed drugs.

Inhibition of rat Bsep was analysed in inverted plasma membrane vesicles prepared from transfected Sf21 insect cells. We quantified the adenosine triphosphate (ATP)-dependent uptake of the probe substrate [3H]-taurocholate by a rapid filtration technique (Stieger et al., 2000). In addition, inhibition of Bsep function in sandwich-cultured primary rat hepatocytes was assessed using cholyllysylfluorescein (CLF) as a probe substrate, by quantifying CLF efflux into canalicular pockets using high content imaging and a Cellomics Arrayscan™ algorithm (manuscript in preparation).

Potent inhibition of rat Bsep activity in vesicles (IC50 < 300 μM) was observed with 22/42 drugs (52%) associated with cholestatic or mixed liver injury in man, 6/25 drugs (23%) associated with hepatocellular liver injury and 5/23 non-hepatotoxic drugs (17%) (Fig. 1A).

A high correlation between potency of rat Bsep inhibition in vesicles and hepatocytes was observed (r2 = 0.79). However, the apparent potency of inhibition was greater in hepatocytes than in vesicles for many of the tested drugs (Fig. 1B). The compounds that exhibited potent inhibition of Bsep in both experimental systems included the withdrawn drugs troglitazone and nefazodone, and bosentan and ketoconazole, both of which have a DILI black box warning.

We conclude that potent Bsep inhibition by drugs correlates with increased risk of cholestatic drug hepatotoxicity in man, and that in vitro evaluation of this liability is useful for DILI hazard identification.
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P27
Oxidative stress via hydrogen peroxide and Menadione does not induce the secretion of IGFBP-5 in primary rat hepatocytes
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Mortality rates are on the increase from liver disease in the developed world, particularly in Scotland. The liver has an enormous capacity for wound repair and regeneration after injury, but early diagnosis is essential. Despite the high number of deaths each year from liver disease, hepatologists still lack relevant diagnostic biomarkers to detect early liver disease.

The aim of this project is to identify a suitable biomarker to detect early liver disease. Insulin-like growth factor binding protein-5 (IGFBP-5) is one such potential biomarker. IGFBP-5 has already been shown to be upregulated in skin fibrosis (Yasuoka et al., 2006a), fibrosis of the lung (Yasuoka et al., 2006b) and has been identified as a genetic marker for intrahepatic cholangiocarcinoma (Blechacz and Gores, 2008). Its role in liver damage is being investigated using an in vitro model of chronic low level toxic insult in primary cultures of rat hepatocytes (male Sprague–Dawley rats, 180–220 g).

The effects of low concentrations of two oxidative toxins (Menadione (0.1, 1, 5 and 10 μM) and hydrogen peroxide (0.01, 0.05,
ing value after 0.01 mM H2O2 exposure was 3.40 compared with controls (3.73 ± 0.03 nmol/well). The correspond-