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Letter to the editor:

QUANTIFICATION OF THREE-DIMENSIONAL STRUCTURES IN LIVER TISSUE: BILE CANALICULAR AND SINUSOIDAL NETWORKS

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Dear Editor,

Unpredicted hepatotoxicity represents the most frequent reason for withdrawal of drugs from the market (Godoy et al., 2013). Therefore, large efforts are currently undertaken to establish improved techniques to predict hepatotoxicity (Shimizu et al., 2009; O'Brien et al., 2006; Okwa et al., 2013; Oloyede et al., 2013; Lu et al., 2013; Godoy et al., 2009). The perhaps most intensive activities in this field of research can be observed in establishment of hepatocyte *in vitro* systems (Godoy et al., 2013; Hewitt et al., 2007; Ilkavets et al., 2013; Hengstler et al., 2012, 2014; Hammad et al., 2013; Hammad, 2013). One of the concepts of this type of *in vitro* research is that stress responses to chemicals are similar *in vitro* and *in vivo*. Therefore, activation of stress response pathways *in vitro* may indicate a potential hazard (Jennings, 2013; Vinken et al., 2014). Although hepatocyte *in vitro* systems have promoted our understanding of mechanisms (Meyer et al., 2011; Schug et al., 2013; Godoy et al., 2010, 2012; Zellmer et al., 2010; Hengstler et al., 2014) it has also become clear that a full replacement of animal experiments for toxicity testing will not be possible within the next one or two decades (Adler et al., 2011; Hammad, 2013). Reasons are difficulties to include xenobiotic metabolism into *in vitro* tests, to model interactions between cell types, to extrapolate from *in vivo* doses to *in vitro* concentrations and to simulate the consequences of long term exposure *in vitro* (Tice et al., 2013; Ghallab, 2013). However, one additional aspect may currently be underestimated: we still know too little about mechanisms of *in vivo* toxicity to establish *in vitro* systems in a way that the most critical *in vivo* processes are recapitulated. Although this may seem paradoxical, progress in replacement of animal experiments currently depends on progress in understanding the mechanisms of toxicity *in vivo*.

Histological alterations often constitute a fingerprint of toxic mechanisms (Hammad et al., 2014). Particularly, liver toxicity often leads to altered tissue microarchitecture (Höhme et al., 2007; Hoehme et al., 2010; Braeuning et al., 2010; Schreiber et al., 2011). Moreover, current studies on liver histology largely rely on image analysis of two-dimensional pictures. However, many aspects of liver tissue architecture such as the complex bile canalicular and sinusoidal networks can be much better quantified using three-dimensional reconstructions. In this context, it represents an important step forward that Dr. Hammad and his team have

established a technique for staining, three-dimensional reconstruction and quantification of liver microarchitecture (Hammad et al., 2014). These protocols which can be applied under routine conditions allow: (1) the simultaneous staining of bile canalicular and sinusoidal networks in approximately 100 µm thick liver tissue blocks; (2) identification of S-phase positive cells whereby the position, size and shape of all individual cells is robustly captured; (3) quantification of key parameters such as hepatocyte volume, the fraction of hepatocytes in contact with neighboring hepatocytes, sinusoids and bile canaliculi. Parameters of the bile canalicular network include the length and branching angles of individual canaliculi, the number of dead end branches and the length of canaliculi per tissue volume. Further, parameters that can be quantified are the radius of the sinusoids, percentage of vessel volume in relation to tissue volume, the branching angle and the length of intersection branches of sinusoids.

Briefly, key parameters of liver tissue microarchitecture can now be quantified routinely. It can be expected that the novel technique will lead to a more precise and robust characterization of hepatotoxicity.

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