

Letter to the editor:

IN VITRO TEST SYSTEMS AND THEIR LIMITATIONS

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Dear editor,

It is well known that huge research networks have been initiated by the EU to establish *in vitro* test systems for toxicity testing (Liebsch et al., 2011; Bolt, 2013; Krug et al., 2013). A letter published in the current issue of this journal discussed the impressive progress made in this field of research in recent years (Hammad, 2013; Knobeloch et al., 2012; Zellmer et al., 2010; Godoy et al., 2009, 2010a, b). Indeed, *in vitro* systems have massively promoted our understanding of mechanisms of toxicity (Valente et al., 2012; Kroll et al., 2012; Vávrová et al., 2011; Clift et al., 2011; Meyer et al., 2011; Schug et al., 2013; Braeuning et al., 2012; Sogorb et al., 2007; Lee et al., 2006). However, critical comments have been published concerning the perspectives for full replacement of animal experiments by *in vitro* systems (Adler et al., 2011). An expert panel confirmed that it may take approximately seven to nine years to replace animal experiments for skin sensitization. For toxicokinetics a timeframe of five to seven years was suggested (Adler et al., 2011). However, for more complex systemic *in vivo* tests, such as repeated dose toxicity, carcinogenicity and reproductive toxicity a time horizon could not yet be given by the expert panel. This opinion confirms a previous analysis of another expert group that came to a similarly pessimistic conclusion (Lilienblum et al., 2008). One reason why it is difficult to establish *in vivo* relevant *in vitro* systems is that isolated and cultivated primary cells usually differ strongly from the corresponding cell type in an organism. For example, when primary hepatocytes are isolated from their normal microenvironment hundreds of genes are up or down regulated (Zellmer et al., 2010). Although not impossible it is technically challenging to establish culture conditions under which at least some of the deregulated genes return to *in vivo* like levels (Godoy et al., 2009, 2010a, b).

Other reasons why the replacement of animal experiments is difficult are (1) difficulties to include xenobiotic metabolism into *in vitro* assays, (2) difficulties to capture interactions between different cell types, (3) problems to extrapolate from *in vivo* doses to *in vitro* concentrations, (4) difficulties in simulating the consequences of long term exposures *in vitro*, (5) difficulties in extrapolating from perturbed pathways or biomarkers *in vitro* to adverse effects *in vivo* (Tice et al., 2013).

However, a further critical aspect may have been underestimated in the recent discussion about possibilities and limitations of *in vitro* systems. Due to their complex architecture and organization organs have possibilities to compensate stress situations that may be lost *in vitro* (Hoehme et al., 2010). In our ongoing research we observed an intriguing example concerning ammonia detoxification (Schliess et al., 2013; Ghallab et al., 2014). In normal liver ammonia is detoxified in a periportal compartment of the liver lobule by the urea cycle, while ammonia that leaks from the periportal to the pericentral region is further metabolized by glutamine synthetase. As long as the interplay between the periportal and pericentral compartments is balanced a periportal expressed enzyme, glutamate dehydrogenase fuels ammonia into the urea cycle by catalyzing a reaction by which glutamate forms α -ketoglutarate and ammonia. After intoxication of mice with either CCl₄ or paracetamol which destroys the pericentral but not the periportal compartment of the liver lobules the capacity of the liver to detoxify ammo-

nia is compromised. Under these stressed conditions glutamate dehydrogenase begins to catalyze an inverse reaction. Instead of producing ammonia, the latter became consumed by the reaction ‘ammonia plus α -ketoglutarate forms glutamate’. Although, we intensively study hepatocyte *in vitro* systems we found it extremely difficult to reproduce this switch of glutamate dehydrogenase *in vitro*. Unfortunately, we are still far from being able to simulate complex situations such as microarchitecture and compartmentation of organs. It may be possible to fully replace animal experiments by *in vitro* systems in future. This will be the case when we (i) have understood all relevant mechanisms of toxicity, interactions between cell types and organs including their compensatory mechanisms, (ii) have established *in vitro* systems that correctly recapitulate all mechanisms. The time horizon for such ambitious goals will be rather centuries than decades. In the meantime we should not neglect *in vivo* research. We will need more knowledge about normally functioning and chemically compromised organs and organisms to establish *in vitro* systems which are more than just *in vitro* artefacts.

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