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 at 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 periportally expressed enzyme, glutamate dehydrogenase fuels ammonia into the urea cycle by catalyzing a reaction by which glutamate forms α-ketoglutorate and ammonia. After intoxication of mice with either CCl_4 or paracetamol which destroys the pericentral but not the periportal compartment of the liver lobules the capacity of the liver to detoxify ammo-

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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-ketoglutorate 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.

REFERENCES

Adler S, Basketter D, Creton S, Pelkonen O, van Benthem J, Zuang V et al. Alternative (non-animal) methods for cosmetics testing: current status and future prospects-2010. Arch Toxicol 2011;85:367-485.

Bolt HM. Developmental neurotoxicity testing with human embryonic stem cell-derived in vitro systems: the novel FP7 ESNATS tests are available. Arch Toxicol 2013;87:5-6.

Braeuning A, Vetter S, Orsetti S, Schwarz M. Paradoxical cytotoxicity of tert-butylhydroquinone in vitro: What kills the untreated cells? Arch Toxicol 2012;86:1481-7.

Clift MJ, Gehr P, Rothen-Rutishauser B. Nanotoxicology: a perspective and discussion of whether or not in vitro testing is a valid alternative. Arch Toxicol 2011;85:723-31.

Ghallab A, Henkel S, Driesch D, Hoehme S, Kuepfer L, Zellmer S et al. Integrated spatial-temporal and metabolic modelling identifies the necessity of alpha-ketoglutarate substitution upon acute liver damage. 2014, in prep.

Godoy P, Hengstler JG, Ilkavets I, Meyer C, Bachmann A, Müller A et al. Extracellular matrix modulates sensitivity of hepatocytes to fibroblastoid dedifferentiation and transforming growth factor beta-induced apoptosis. Hepatology 2009;49:2031-43.

Godoy P, Schug M, Bauer A, Hengstler JG: Reversible manipulation of apoptosis sensitivity in cultured hepatocytes by matrix-mediated manipulation of signaling activities. Methods Mol Biol 2010a;640: 139-55.

Godoy P, Lakkapamu S, Schug M, Bauer A, Stewart JD, Bedawi E et al. Dexamethasone-dependent versus -independent markers of epithelial to mesenchymal transition in primary hepatocytes. Biol Chem 2010b;391:73-83.

Hammad S. Advances in 2D and 3D in vitro systems for hepatotoxicity testing. EXCLI J 2013;12:993-6.

Hoehme S, Brulport M, Bauer A, Bedawy E, Schormann W, Hermes M et al. Prediction and validation of cell alignment along microvessels as order principle to restore tissue architecture in liver regeneration. Proc Natl Acad Sci U S A 2010;107:10371-6.

Knobeloch D, Ehnert S, Schyschka L, Büchler P, Schoenberg M, Kleeff J et al. Human hepatocytes: isolation, culture, and quality procedures. Methods Mol Biol 2012;806:99-120.

Kroll A, Pillukat MH, Hahn D, Schnekenburger J. Interference of engineered nanoparticles with in vitro toxicity assays. Arch Toxicol 2012;86(7):1123-36.

Krug AK, Kolde R, Gaspar JA, Rempel E, Balmer NV, Meganathan K et al. Human embryonic stem cell-derived test systems for developmental neurotoxicity: a transcriptomics approach. Arch Toxicol 2013;87:123-43.

Lee MJ, Ayaki H, Goji J, Kitamura K, Nishio H. Cadmium restores in vitro splicing activity inhibited by zinc-depletion. Arch Toxicol 2006;80:638-43.

Liebsch M, Grune B, Seiler A, Butzke D, Oelgeschläger M, Pirow R et al. Alternatives to animal testing: current status and future perspectives. Arch Toxicol 2011;85:841-58.

Lilienblum W, Dekant W, Foth H, Gebel T, Hengstler JG, Kahl R et al. Alternative methods to safety studies in experimental animals: role in the risk assessment of chemicals under the new European Chemicals Legislation (REACH). Arch Toxicol 2008;82:211-36.

Meyer C, Godoy P, Bachmann A, Liu Y, Barzan D, Ilkavets I et al. Distinct role of endocytosis for Smad and non-Smad TGF-β signaling regulation in hepatocytes. J Hepatol 2011;55:369-78.

Schliess F, Hoehme S, Henkel S, Ghallab A, Driesch D, Böttger J et al. Integrated metabolic spatial-temporal models for prediction of ammonia metabolism during liver damage and regeneration. Hepatology 2013, in revision.

Schug M, Stöber R, Heise T, Mielke H, Gundert-Remy U, Godoy P et al. Pharmacokinetics explain in vivo/in vitro discrepancies of carcinogen-induced gene expression alterations in rat liver and cultivated hepatocytes. Arch Toxicol 2013;87:337-45.

Sogorb MA, Alvarez-Escalante C, Carrera V, Vilanova E. An in vitro approach for demonstrating the critical role of serum albumin in the detoxication of the carbamate carbaryl at in vivo toxicologically relevant concentrations. Arch Toxicol 2007;81:113-9.

Tice RR, Austin CP, Kavlock RJ, Bucher JR. Improving the human hazard characterization of chemicals: a Tox21 update. Environ Health Perspect 2013;121:756-65.

Valente MJ, Henrique R, Vilas-Boas V, Silva R, Bastos Mde L, Carvalho F et al. Cocaine-induced kidney toxicity: an in vitro study using primary cultured human proximal tubular epithelial cells. Arch Toxicol 2012;86:249-61.

Vávrová A, Popelová O, Stěrba M, Jirkovský E, Hašková P, Mertlíková-Kaiserová H et al. In vivo and in vitro assessment of the role of glutathione antioxidant system in anthracycline-induced cardiotoxicity. Arch Toxicol. 2011 May;85(5):525-35. doi: 10.1007/s00204-010-0615-8.

Zellmer S, Schmidt-Heck W, Godoy P, Weng H, Meyer C, Lehmann T et al. Transcription factors ETF, E2F, and SP-1 are involved in cytokine-independent proliferation of murine hepatocytes. Hepatology 2010;52:2127-36.