EXCLI Journal 2014;13:1292-1294 – ISSN 1611-2156
Received: December 17, 2014, accepted: December 17, 2014, published: December 18, 2014

Guest editorial:

CONCEPTS OF PREDICTIVE TOXICOLOGY

Raymond Reif

Leibniz-Institut für Arbeitsforschung an der TU Dortmund,
Leibniz Research Centre for Working Environment and Human Factors (IfADo),
Ardeystrasse 67, 44139 Dortmund, Germany; reif@ifado.de

Currently, much effort is invested into the development of non-animal testing strategies to identify the potential of compounds to induce systemic toxicity (Hammad, 2013; Stewart and Marchan, 2012). Organotypical *in vitro* systems are particularly popular in the fields of kidney (Limonciel et al., 2012; Jennings et al., 2012; Valente et al., 2012), heart (Maayah et al., 2014; Bonifacio et al., 2014), liver (Grinberg et al., 2014; Godoy et al., 2013; Schug et al., 2013) and developmental toxicity (Weng et al., 2014; Waldmann et al., 2014; Krug et al., 2013). However, it is also clear that *in vitro* systems represent valuable tools to study certain mechanisms and endpoints but do not reach the complexity of organs or organisms (Ghallab, 2013).

Recently, Daston et al. (2014) published a concept, how future research on non-animal methodology should be designed to overcome current limitations. The authors recommend two complementary and interconnecting concepts. A first work stream should focus on toxicity characterization. Here, critical biological targets and mechanisms leading to toxic effects should be elucidated based on *in vitro* systems. For this purpose methods such as high-throughput and high content screening and computational modelling will be applied (Daston et al., 2014). A second work stream should focus on translation into regulation. Specific aims are for example methods for grouping, read-across strategies and *in vitro* methods to derive no-effect levels. In recent years much has been written about grouping strategies and general concepts to improve chemical risk evaluation (Geenen et al., 2012; Kalkhof et al., 2012; Keller et al., 2009; Renwick, 2004; Zbinden, 1993; Gebel et al., 2014; Calabrese, 2013). The present concept paper of Daston and colleagues (2014) belongs certainly to the most fundamental papers in this field and is a must-read for anyone interested in predictive toxicology and alternative methods. However, the authors neglect one major limitation of their strategy: The concept may lead to reasonable predictions for chemicals with unspecific mechanisms of action, meaning that many mechanisms are simultaneously active that lead to the breakdown of cellular functions. However, the concept may fail for highly specific mechanisms of action. The reason for this limitation is that Daston et al. (2014) in agreement with the SEURAT concept recommend focusing on ‘critical biological targets’ in *in vitro* systems only (Jennings et al., 2014). This bears the risk of establishing an illusory *in vitro* world which lacks critical components of real organs or organisms. Let us assume a compound specifically inhibits reabsorption of bile salts in cholangiocytes in bile ducts. How should this mechanism be recognized in an *in vitro* system that contains hepatocytes only? Moreover, it cannot be excluded that a compound may alter kidney cells in a way that triggers the attack of immue cells. Can we be sure that this specific mechanism would be identified in an *in vitro* system containing renal proximal tubular epithelial cells only? A research program to *in vitro* systems only has a high probability to

fail. Therefore, a third work stream is painfully missing in the concept of Daston et al. (2014); namely research that systematically compares mechanisms of toxicity *in vitro* and *in vivo*. Do the currently available *in vitro* systems really recapitulate the mechanisms that finally lead to adverse effects *in vivo*? Finally, it should not be ignored that many mechanisms leading to toxicity *in vivo* are far from being fully understood. Further research is needed to identify key mechanisms of toxicity *in vivo* to be able to establish *in vitro* systems recapitulating these mechanisms. Although it may seem paradox: the successful development of non-animal methodology requires animal experiments.

REFERENCES

Bonifacio A, Mullen PJ, Mityko IS, Navegantes LC, Bouitbir J, Krähenbühl S. Simvastatin induces mitochondrial dysfunction and increased atrogin-1 expression in H9c2 cardiomyocytes and mice in vivo. Arch Toxicol. 2014 [Epub ahead of print].

Calabrese EJ. Origin of the linearity no threshold (LNT) dose-response concept. Arch Toxicol. 2013; 87:1621-33

Daston G, Knight DJ, Schwarz M, Gocht T, Thomas RS, Mahony C, et al. SEURAT: Safety Evaluation Ultimately Replacing Animal Testing-Recommendations for future research in the field of predictive toxicology. Arch Toxicol. 2014 [Epub ahead of print].

Gebel T, Foth H, Damm G, Freyberger A, Kramer PJ, Lilienblum W, et al. Manufactured nanomaterials: categorization and approaches to hazard assessment. Arch Toxicol. 2014; 88:2191-211.

Geenen S, Taylor PN, Snoep JL, Wilson ID, Kenna JG, Westerhoff HV. Systems biology tools for toxicology. Arch Toxicol. 2012;86:1251-71.

Ghallab A. *In vitro* test systems and their limitations, EXCLI J 2013;12:1024-6.

Godoy P, Hewitt NJ, Albrecht U, Andersen ME, Ansari N, Bhattacharya S, et al. Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. Arch Toxicol. 2013;87:1315-530.

Grinberg M, Stöber RM, Edlund K, Rempel E, Godoy P, Reif R, et al. Toxicogenomics directory of chemically exposed human hepatocytes. Arch Toxicol. 2014;88:2261-87.

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

Jennings P, Weiland C, Limonciel A, Bloch KM, Radford R, Aschauer L, et al. Transcriptomic alterations induced by Ochratoxin A in rat and human renal proximal tubular in vitro models and comparison to a rat in vivo model. Arch Toxicol. 2012;86:571-89.

Jennings P, Schwarz M, Landesmann B, Maggioni S, Goumenou M, Bower D, et al. SEURAT-1 liver gold reference compounds: a mechanism-based review. Arch Toxicol. 2014;88:2099-133.

Kalkhof H, Herzler M, Stahlmann R, Gundert-Remy U. Threshold of toxicological concern values for non-genotoxic effects in industrial chemicals: re-evaluation of the Cramer classification. Arch Toxicol. 2012;86:17-25.

Keller D, Krauledat M, Scheel J. Feasibility study to support a threshold of sensitization concern concept in risk assessment based on human data. Arch Toxicol. 2009;83:1049-60.

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.

Limonciel A, Wilmes A, Aschauer L, Radford R, Bloch KM, McMorrow T, et al. Oxidative stress induced by potassium bromate exposure results in altered tight junction protein expression in renal proximal tubule cells. Arch Toxicol. 2012;86:1741-51.

Maayah ZH, Ansari MA, El Gendy MA, Al-Arifi MN, Korashy HM. Development of cardiac hypertrophy by sunitinib *in vivo* and *in vitro* rat cardiomyocytes is influenced by the aryl hydrocarbon receptor signaling pathway. Arch Toxicol. 2014;88:725-38.

Renwick AG. Toxicology databases and the concept of thresholds of toxicological concern as used by the JECFA for the safety evaluation of flavouring agents. Toxicol Lett. 2004;149:223-34.

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.

Stewart JD, Marchan R. Current developments in toxicology. EXCLI J 2012;11:692-702.

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.

Waldmann T, Rempel E, Balmer NV, König A, Kolde R, Gaspar JA, et al. Design principles of concentration-dependent transcriptome deviations in drug-exposed differentiating stem cells. Chem Res Toxicol. 2014;27:408-20.

Weng MK, Natarajan K, Scholz D, Ivanova VN, Sachinidis A, Hengstler JG, et al. Lineage-specific regulation of epigenetic modifier genes in human liver and brain. PLoS One. 2014;9(7):e102035.

Zbinden G. The concept of multispecies testing in industrial toxicology. Regul Toxicol Pharmacol. 1993;17:85-94.