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EDITORIAL

Standard compounds for establishment of in vitro test systems

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January 1, 2011, rang in the start of the European Union's ambitious initiative to 'replace in vivo repeated dose systemic toxicity testing.' SEURAT-1, shortened for Safety Evaluation Ultimately Replacing Animal Testing, was launched as the first step in the gargantuan FP7 research initiative to change the way that chemical safety is assessed by providing more accurate in vitro testing methods and improved predictive tools. In the present issue of the *Archives of Toxicology*, the editors would like to specially highlight the review article by member scientists of SEURAT-1 who comprehensively summarized SEURAT-1 liver gold standard compounds (Jennings et al. 2014). The authors have selected a particularly useful set of compounds to support the development of in vitro systems for the identification of hepatotoxic compounds, while simultaneously reviewing their known mechanisms of action. Briefly, the following principles were addressed:

- The two major mechanisms of hepatotoxicity—cytotoxicity and dysregulation of lipid metabolism.
- Groups of well-characterized compounds with alkylating and oxidizing activities as cytotoxicity can be non-specifically induced by chemically reactive compounds.
- Standard compounds that act via highly specific cellular targets, such as inhibitors of oxidative phosphorylation or ligands of nuclear receptors. Moreover, compounds were selected that act only on single factors within lipid metabolism pathways.
- In addition to the highly specific compounds, promiscuous chemicals were also reviewed that initiate several molecular events leading to both cytotoxicity and compromised lipid metabolism.

The development of alternative methods to experimental animals in safety studies is at the leading edge of the current toxicological research (Seiler et al. 2011; Driessen et al. 2013; Lilienblum et al. 2008; Krug et al. 2013; Aritomi et al. (2014); Dias da Silva et al. (2013); Guo et al. (2014); Knobeloch et al. (2012)). Relevant in vitro systems for hepatotoxicity studies are of particular importance (Godoy et al. 2013; Schyschka et al. 2013; Rodrigues et al. 2013; Tolosa et al. 2013; Messner et al. 2013; Hewitt et al. 2007; Ufelmann and Schrenk 2014). Therefore, the well-chosen set of compounds that represent known mechanisms of hepatotoxicity that are presented by Jennings et al. (2014) will facilitate the systematic establishment of in vitro systems for hepatotoxicity. Nevertheless, the bar could be raised even higher in the future.

One pertinent question is how comprehensive testing of the recommended compounds brings us beyond the current state of the art. We will certainly learn whether the gold standard compounds are able to activate certain mechanisms of toxicity in the exposed cells. Specific patterns of deregulated genes or proteins will be associated with specific compounds and will provide evidence of the involved biological processes. At best, this strategy will allow hazard identification; however, toxicology requires quantitative information. To aid regulatory processes, in vitro systems must ideally predict in vivo doses that are safe and those that would result in adverse effects. Simply having the knowledge that a particular concentration (often very high) activates a characteristic mechanism of toxicity in vitro will not be sufficient.

Despite intensive research in the field of alternative methods, some challenges are still currently underestimated. For example, a response induced by a compound in

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a cultivated cell in vitro, e.g., a primary hepatocyte, is not automatically comparable to the effect caused by the same concentration used on the equivalent target cell in vivo, e.g., a hepatocyte in the liver. Although some metabolism and stress response-associated genes have shown a good in vivo/in vitro correlation (Heise et al. 2012), the culture conditions may massively alter the responsiveness of cells to chemicals, e.g., by activating anti-apoptotic mechanisms (Godoy et al. 2009, 2010). Therefore, a careful 'translation' of observations in vitro to the in vivo situation is required. Furthermore, an organism may have compensatory mechanisms that cannot become active in vitro, and specific mechanisms, such as the immune response, may be lacking in the current in vitro systems.

Progress requires a next generation of 'quantitative' gold standard compounds with accompanying data on blood concentrations in humans that are associated with increased risk of hepatotoxicity and those that are considered safe. Ideally, the concentrations in the target cells, e.g., the hepatocytes, should also be known; information that in humans is usually only obtained by PBPK modeling. Such a list of some hundreds of 'quantitative gold standard compounds' with known concentration ranges of 'hepatotoxic risk' plus information on the expected mechanism would enormously support the evaluation of in vitro systems. Although the current review by Jennings and colleagues has clearly raised the bar—the hunt for gold standard compounds for evaluating in vitro systems of toxicity has only just begun.

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