Towards an integrated in vitro strategy for repeated dose toxicity testing

Tamara Vanhaecke · Marleen Pauwels ·
Mathieu Vinken · Liesbeth Ceelen ·
Vera Rogiers

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Currently, there is clear political incentive in the EU to minimize the number of animals involved in the chemical risk assessment process, particularly holding true for the cosmetics sector. Stringent requirements of full replacement of animals in safety testing are laid down in the EU Cosmetics Directive 76/768/EEC by the implementation of the 7th Amendment (2003/15/EC), setting a rigid time frame for the availability of valid 3R-alternative methods for the safety testing of cosmetic products and their ingredients (EU 2003). An important step forward to achieve this goal has recently been made through a joint initiative by DG Research and Colipa (The European Cosmetics Association). A cluster of calls was launched under the FP7 health programme specifically addressing the replacement of repeated dose systemic toxicity testing in human safety assessment. As such, 6 large-scale collaborative research projects started from January 2011 onwards, covering key themes related to human stem cell technology (SCREEN-TOX), organ-simulating devices (HeMiBio), human-relevant biomarker detection (DETECTIVE), computational modelling (COSMOS), systems biology (NOTOX) and

integrated data analysis (TOXBANK). An umbrella coordination and support action project (COACH) is responsible for monitoring progress and potential gaps occurring throughout the 5-year running period. A major task, however, will be to streamline and combine the outcomes of the different projects in order to establish a realistic in vitro strategy to assess repeated dose toxicity.

As in all projects the liver is involved as target organ, one might expect that hepatotoxicity could be used as a test case. This would be of particular interest since it is the major type of toxicity observed during in vivo repeated dose toxicity testing for cosmetic ingredients (Pauwels et al. 2009) and pharmaceutical compounds (Russmann et al. 2009).

Figure 1 demonstrates how such an in vitro testing strategy could look, taking into consideration, the current approach of quantitative risk assessment for cosmetic ingredients using data derived from animal studies. Thus, a margin of safety (MoS) of at least 100 is applied for intraspecies and interspecies variation using the following equation:

$$\text{MoS} = \frac{\text{NOAEL (no observable adverse effect level)}}{\text{systematic exposure dosage}} \geq 100$$

In the proposed strategy, a computed NOAEL value, preferably derived from human data (hNOAEL_c), could be used for the compound of interest. This estimated in vivo dosage could be obtained via processing of the compound concentration (hNOAEC) that does not cause any adverse effect using an in vitro model. For that purpose, physiologically based pharmacokinetic (PBPK) computational modelling can be applied taking into account an appropriate uncertainty factor for the in vitro/in vivo extrapolation. The hNOAEC value of the compound of interest could, for example,

T. Vanhaecke (✉) · M. Pauwels · M. Vinken · L. Ceelen ·
V. Rogiers
Department of Toxicology, Vrije Universiteit Brussel,
Laarbeeklaan 103, 1090 Brussels, Belgium
e-mail: tamara.vanhaecke@vub.ac.be

M. Pauwels
e-mail: mnpauwel@vub.ac.be

M. Vinken
e-mail: mvinken@vub.ac.be

L. Ceelen
e-mail: lceelen@vub.ac.be

V. Rogiers
e-mail: vrogiere@vub.ac.be

Fig. 1 Proposal for an integrated approach for the in vitro assessment of repeated dose hepatotoxicity. The contributions of the different partners involved in the ongoing EU-Colipa initiative are indicated. (hNOAEC human no observable adverse effect concentration,

hNOAEL human oral NOAEL, hNOAEL_c computed hNOAEL, NOAEL no observable adverse effect level, QSAR quantitative structure–activity relationship)

be derived from the exposure of human hepatocyte cultures for 2 weeks while monitoring relevant biomarkers at different compound concentrations. As human hepatocytes are scarce, the project cluster foresees that they will be generated through stem cell technology (preferably in 3D-configuration culture). Also the relevant biomarkers selection should be the result of the combination of efforts of different projects, namely a combination of parameter analysis, compound selection and integrated 'omics' and functional analysis, outlined in Fig. 1.

When the prototype design proposed for the liver would be successful, it could be equally applied to other organs (e.g. heart, kidney and lungs), with the ultimate goal to enable the selection of the lowest hNOAEL_c and thereby also of the most sensitive target organ. As such, the integrated in vitro strategy proposed becomes more complex, though it provides a better reflection of the current in vivo approach for repeated dose toxicity testing of chemical

substances. Anyhow, attempts to develop an in vitro alternative to repeated dose toxicity testing in animals is a crucial step forward towards a more animal-friendly way of protecting human health.

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