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Review

Insights on *in vitro* models for safety and toxicity assessment of cosmetic ingredients

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ABSTRACT

According to the current European legislation, the safety assessment of each individual cosmetic ingredient of any formulation is the basis for the safety evaluation of a cosmetic product. Also, animal testing in the European Union is prohibited for cosmetic ingredients and products since 2004 and 2009, respectively. Additionally, the commercialization of any cosmetic products containing ingredients tested on animal models was forbidden in 2009. In consequence of these boundaries, the European Centre for the Validation of Alternative Methods (ECVAM) proposes a list of validated cell-based *in vitro* models for predicting the safety and toxicity of cosmetic ingredients. These models have been demonstrated as valuable and effective tools to overcome the limitations of animal *in vivo* studies. Although the use of *in vitro* cell-based models for the evaluation of absorption and permeability of cosmetic ingredients is widespread, a detailed study on the properties of these platforms and the *in vitro-in vivo* correlation compared with human data are required. Moreover, additional efforts must be taken to develop *in vitro* models to predict carcinogenicity, repeat dose toxicity and reproductive toxicity, for which no alternative *in vitro* methods are currently available. This review paper summarizes and characterizes the most relevant *in vitro* models validated by ECVAM employed to predict the safety and toxicology of cosmetic ingredients.

Contents

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1. Introduction

Nowadays, it is generally accepted that the safety assessment of each cosmetic ingredient is the basis for the safety evaluation of a cosmetic product. According to the European Cosmetic Regulation 1223/2009, any cosmetic product must be secure for human health under normal or foreseeable conditions of use (European Parliament, 2009). The safeness must be guaranteed by an independent safety assessor with demonstrated qualification. The assessment of the toxicological potential lays on distinct toxicity studies, specific for different toxicological endpoints, representing the primary step in the hazard evaluation of a cosmetic ingredient. Since 2004, animal tests are prohibited in the European Union (EU) for cosmetic products, being also banned in 2009 for cosmetic ingredients (European Comission, 2003). In 2009, the commercialization of cosmetic products containing ingredients tested on animals was forbidden in the European market. For the most complex human health effects (carcinogenicity, skin sensitization, reproductive toxicity and toxicokinetics), an extension until March 2013 was agreed for the marketing ban (European Comission, 2003).

The Scientific Committee on Consumer Safety (SCCS) of the EU was the responsible, in 2015, for the recommendation of risk assessment guidelines for the safety evaluation of cosmetic ingredients and products (SCCS, 2015). These safety requirements are listed in the "Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation", and include the following parameters: (a) Acute Toxicity; (b) Corrosivity and Irritation; (c) Skin Sensitization; (d) Dermal/Percutaneous Absorption; (e) Repeated Dose Toxicity; (f) Reproductive Toxicity; (g) Mutagenicity/Genotoxicity; (h) Carcinogenicity; (i) Toxicokinetics Studies; (j) Photo-induced Toxicity and (k) Human Data (SCCS, 2015).

Nevertheless, it is still anticipated by the scientific community up to ten years for the complete replacement of the existing *in vivo* animal tests used for the skin sensitization, lung absorption or toxicokinetics. Following any potential systemic absorption of cosmetic ingredient, the timeframe is set between 5 and 7 years to develop models to calculate renal/biliary excretion (Adler et al., 2011). Regarding the systemic toxicological endpoints of carcinogenicity, repeat dose or reproductive toxicity, the time needed for the full replacement has not been clearly estimated (Adler et al., 2011). For these endpoints, no alternative *in vitro* methods are available, although the diagnostic to address the systemic endpoints impacts is extensive (Basketter et al., 2012).

In this review paper, the most relevant cell-based *in vitro* models, validated by the European Centre for the Validation of Alternative Methods (ECVAM), to predict the safety and toxicity of cosmetic ingredients are detailed, with special focus on those now available for commercialization. Also, it is provided a critical analysis on the rationale behind the development of these models and, when the pertinent animal/human data are available, the assessment of *in vitro-in vivo* correlation.

2. *In vitro* cell models

2.1. Toxicokinetics

Toxicokinetics has been defined as the quantitation of the time course of toxicants in the body during the processes of ADME or clearance. The toxicokinetic process ends with a biological effective dose of the toxicant, thus becoming fundamental for the human risk assessment without animal use (Bessems et al., 2014). Still, beyond toxicokinetics, ADME data are critical, as the safety assessment of cosmetics is based on alternative methods, such as *in silico* and *in vitro* models. Theoretical models for predicting ADME properties play progressively more important roles in drug and cosmetics development process. The above mentioned toxicokinetic/pharmacokinetic (PBPK/PBTK) models can simulate the biological profile of any chemical (and its metabolites) in organs and blood, and thus, a compound-specific kinetic scenario of ADME (Gajewska et al., 2014).

The use of PBTK models has significantly improved in recent years based on the precise simulations of *in vivo* ADME processes in living organisms, oppositely to conventional kinetic models (Gajewska et al., 2015; Jónsdóttir et al., 2016). These models are being increasingly used as an effective tool to design toxicology experiments and conducting essential extrapolations for risk assessments, particularly the time-dependent concentration of compounds at the organ level (Krishnan and Peyret, 2009; Gajewska et al., 2015). However, there are only few PBTK platforms freely-available, with limitations for their use (Bessems et al., 2014). Examples include unknown or incomplete applicability domains and lack of highthroughput tools to measure penetration of barriers, partitioning between blood and tissues and metabolic clearance (Bessems et al., 2014). Curiously, the separate TK and biotransformation studies are not part of current cosmetic dossiers, except for the *in vitro* dermal absorption studies. Also, in cosmetic field, specific supplementary studies are only performed in the case of TK decrease or toxicodynamic underlying the minimal Margin of Safety (MoS) value of 100 and/or to elucidate mechanisms.

For cosmetics, ECVAM had only validated one model to evaluate absorption properties of drugs in the ADME process (OECD, 2004a). The model uses human skin static or flow-through diffusion cell models, with standardized *in vitro* conditions and rat, pig or human skin. This accepted OECD test guideline (OECD, 2004a) is not only for cosmetic testing but also for regulatory context. Nonetheless, this guideline is not very specific and additional recommendations for the conduct of dermal absorption studies were provided by authorities (SCCS, 2015). Indeed, for dermal absorption, key factors