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A tiered approach to the use of alternatives to animal testing for the safety assessment of cosmetics: Eye irritation

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ABSTRACT

The need for alternative approaches to replace the *in vivo* rabbit Draize eye test for evaluation of eye irritation of cosmetic ingredients has been recognised by the cosmetics industry for many years. Extensive research has lead to the development of several assays, some of which have undergone formal validation. Even though, to date, no single *in vitro* assay has been validated as a full replacement for the rabbit Draize eye test, organotypic assays are accepted for specific and limited regulatory purposes. Although not formally validated, several other *in vitro* models have been used for over a decade by the cosmetics industry as valuable tools in a weight of evidence approach for the safety assessment of ingredients and finished products. In light of the deadlines established in the EU Cosmetics Directive for cessation of animal testing for cosmetic ingredients, a COLIPA scientific meeting was held in Brussels on 30th January, 2008 to review the use of alternative approaches and to set up a decision-tree approach for their integration into tiered testing strategies for hazard and safety assessment of cosmetic ingredients and their use in products. Furthermore, recommendations are given on how remaining data gaps and research needs can be addressed.

1. Introduction

According to the Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals Test No. 405: acute eye irritation/corrosion, eye irritation is defined as "... the production of changes in the eye following application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application". The same guideline defines eye corrosion as "... the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application" (OECD TG 405, 2002). Different regulatory systems exist, e.g., within the European Union (EU) (EU, 2004), United States and on a more global basis (UN, 2003) which classify substances based on the severity and persistence of the eye responses (cornea, iris and conjunctiva) that they produce. Such classifications translate into labelling of the substance and for products where required by legislation.

In general, topical eye irritants cause local effects on the front structures of the eye e.g., cornea, conjunctiva, iris and lachrymal system. The extent of involvement of these different ocular structures in irritation is a reflection of the severity of the response. Typically, slight irritants produce primarily conjunctival effects with little or no corneal involvement. While conjunctival responses generally precede corneal responses, corneal injury is associated with

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moderate and severe irritation responses. Research into the *in vivo* mechanistic basis for ocular irritation using different chemical classes comprising surfactants (anionic, cationic, and non-ionic), acids, alcohols, aldehydes, alkalis and bleaches have shown that depth of injury to the cornea, in the early hours after exposure, is predictive of the eventual degree and duration of the ocular lesions in the rabbit (Maurer et al., 2002; Jester, 2006). This research demonstrated that slight irritants tend to affect only the superficial corneal epithelium, mild and moderate irritants affect epithelium and superficial stroma whilst highly moderate and severe irritants affect deeper layers of the stroma (and possibly the endothelium). In turn, the depth of injury is also related to the eventual degree and recovery of the injury. Common mechanisms of injury causing acute effects include membrane lysis, protein coagulation, saponification and action on macromolecules. Chemicals that react with nucleic acids, mitochondrial proteins, or other cellular targets often show a longer latency period between exposure and maximum manifestation of damage to the cornea (Maurer et al., 2002; Jester, 2006).

Cosmetics may come into contact with the eye under conditions of intended use or accidental exposure (e.g., in the case of mascaras and shampoos, respectively). Both scenarios need to be evaluated in a proper safety assessment, as stipulated in the EU Cosmetics Directive (EU, 1976). Due to this potential exposure, it is essential to assess the ocular safety of cosmetic ingredients and/or final cosmetic products. The rabbit Draize eye test (OECD TG 405, 2002) is globally accepted as the standard regulatory method for evaluating the eye irritation potential of substances and has been used for several decades. An extensive number of *in vitro* models have been developed and proposed as alternatives to the rabbit Draize eye test. A overview of these methods is available in a comprehensive review published by Eskes et al. (2005). Several of these *in vitro* assays have been included in six major validation or evaluation studies (EC/HO (Balls et al., 1995), COLIPA (Brantom et al., 1997), BGA/BMBF (Spielmann et al., 1993, 1996), CTFA (Gettings et al., 1991, 1994, 1996), IRAG (Bradlaw et al., 1997) and MHW/JCIA (Ohno et al., 1994)) that took place between 1991 and 1997. A review of these studies (Balls et al., 1999) concluded that despite good reproducibility and sensitivity of several of the *in vitro* assays for ocular irritation, the predictive performance of each individual assay was not sufficient to fully replace the rabbit Draize eye test. Despite this, organotypic assays (models that resemble the *in vivo* situation in 3-D form or function or both) are widely used for specific, limited regulatory purposes. The Bovine Corneal Opacity and Permeability (BCOP),[1] Isolated Chicken Eye (ICE), Isolated Rabbit Eye (IRE) and the Hen's Egg Test on the Chorio-Allantoic Membrane (HET-CAM) have been officially accepted since 2004 by European authorities for the classification and labelling of severe eye irritants. More recently, the European Centre for the Validation of Alternative Methods (ECVAM) Scientific Advisory Committee (ESAC) issued statements of scientific validity for BCOP and ICE as screening tests for identification of ocular corrosives and severe eye irritants (ECVAM, 2007). These statements support the outcome of the Interagency Co-ordinating Committee for the Validation of Alternative Methods (ICCVAM) Background Review Document activities for these organotypic assays (ICCVAM, 2006). In order to identify irritants over the entire potency range for all chemical classes, it is generally accepted that a battery of alternative assays will be required. Furthermore, the cosmetics industry has a need for *in vitro* assays that provide greater resolution and precision in the mild to very mild range of eye irritancy than are offered by the standard rabbit Draize eye test.

On 11 March 2009, two bans entered into force concerning animal testing related to cosmetics products in the European Union. Both were decided in 2003 in the context of the 7th amendment to the Cosmetics Directive (EU 2003), which, amongst other purposes, aims at ensuring the safety of ingredients used in cosmetic products. A first ban concerns animal testing itself to assess the safety of ingredients. A second ban prohibits the sale of cosmetic products containing ingredients tested on animals. This ban is progressive, until it becomes a complete ban in March 2013 taking into account scientific progress being made regarding repeat dose tests for which alternative methods do not yet exist. The impact of the ban on the use of alternative assays to replace animal tests for the assessment of eye irritation after March 2009 was analysed at a COLIPA scientific meeting organised by its Safety Assessment and Eye Irritation Project Teams in Brussels on 30th January, 2008. Participants included safety experts from a number of cosmetic companies. Decision trees for safety assessment were developed using the outcome of the discussions held during the meeting, in which tiered testing strategies and the use of weight-of-evidence (WoE) were considered major principles. Gaps and hurdles were also identified and recommendations for further activities were developed.

2. Results and discussion

2.1. Current alternative approaches to the assessment of eye irritation

Current safety assessment practices make routine use of tiered testing strategies based on a WoE approach. WoE approaches have long been in use and have also been investigated by ECVAM in the context of validation (Balls et al., 2006). The principle is that all available information is considered in the assessment, in this case of eye irritation. Such information may include, for example:

- Physicochemical properties.
- Historical *in vivo* animal data.
- *In vitro* data.
- Human data (clinical and post-market surveillance).
- Exposure.

If the information which is initially available is considered insufficient, a tiered testing strategy is pursued that allows for the generation of additional data. Important elements may include read-across approaches based on chemical domain (OECD Application Toolbox (www.oecd.org)). Integrated testing strategies have been applied in the chemical sector, and were recently re-evaluated in the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) context (Grindon et al., 2008), as well as the application of WoE approaches (e.g., OSIRIS project) (van Leeuwen et al., 2007).

[1] BCOP, Bovine Corneal Opacity and Permeability; BfR, Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment); BGA, Bundesgesundheitsamt (German Department of Research and Technology); BMBF, Bundesministerium für Bildung und Forschung (German Federal Ministry of Education and Research); COLIPA The European Cosmetic Association; CTFA, Cosmetic, Toiletry and Fragrance Association; DSS, Decision Support System; EC/HO, European Commission/British Home Office; ECVAM, European Centre for the Validation of Alternative Methods; EPAA, European Partnership on Alternative Approaches to Animal Testing; ESAC, ECVAM Scientific Advisory Committee; EU, European Union; GHS, Globally Harmonised System; HCE, Human corneal epithelium; HET-CAM, Hen's Egg Test on the Chorio-Allantoic Membrane; ICCVAM, Interagency Co-ordinating Committee for the Validation of Alternative Methods; ICE, Isolated Chicken Eye; IRAG, Interagency Regulatory Alternatives Group; MHW/JCIA, (Japanese) Ministry of Health and Welfare/Japanese Cosmetic Industry Association; NC, not classified; OECD, Organisation for Economic Co-operation and Development; (Q)SAR, (Quantitative) Structure Activity Relationship; Reconstructed human Tissue (RhT); REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; TTC, Threshold of Toxicological Concern; WoE, weight of evidence.