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A tiered approach to the use of alternatives to animal testing for the safety assessment of cosmetics: Genotoxicity. A COLIPA analysis

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ABSTRACT

For the assessment of genotoxic effects of cosmetic ingredients, a number of well-established and regulatory accepted *in vitro* assays are in place. A caveat to the use of these assays is their relatively low specificity and high rate of false or misleading positive results. Due to the 7th amendment to the EU Cosmetics Directive ban on *in vivo* genotoxicity testing for cosmetics that was enacted March 2009, it is no longer possible to conduct follow-up *in vivo* genotoxicity tests for cosmetic ingredients positive in *in vitro* genotoxicity tests to further assess the relevance of the *in vitro* findings. COLIPA, the European Cosmetics Association, has initiated a research programme to improve existing and develop new *in vitro* methods. A COLIPA workshop was held in Brussels in April 2008 to analyse the best possible use of available methods and approaches to enable a sound assessment of the genotoxic hazard of cosmetic ingredients. Common approaches of cosmetic companies are described, with recommendations for evaluating *in vitro* genotoxins using non-animal approaches. A weight of evidence approach was employed to set up a decision-tree for the integration of alternative methods into tiered testing strategies.

1. Introduction

In vitro tests form an essential part of the assessment of genotoxicity and provide information on three major genetic endpoints, namely (1) mutagenicity at a gene level, (2) chromosome breakage and/or rearrangements (clastogenicity), and (3) numerical chromosome aberrations (CA)[2] (aneugenicity) (SCCP, 2006a,b; Mueller et al., 2003; Dearfield et al., 2002; COM, 2000). In the past, *in vivo*

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[2] *Abbreviations used:* ADME, absorption, distribution, metabolism and excretion; CA, chromosome aberration; CHO, Chinese hamster ovary; COLIPA, The European Cosmetic Association; DEREK, deductive estimation of risk from existing knowledge; ECVAM, European Centre for the Validation of Alternative Methods; HPRT, hypoxanthine-guanine phosphoribosyl transferase; ICH, The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; K_M, Michaelis constant; MLA, mouse lymphoma assay; MNT, micronucleus test; MPs, misleading positives; OECD, organisation for economic co-operation and development; RDT, repeated dose toxicity; REACh, regulation, evaluation, authorisation of chemicals; RS, reconstructed skin; RSMN, reconstructed skin micronucleus test; SCCP, Scientific Committee on Consumer Products; SCCS, Scientific Committee on Consumer Safety (formerly SCCP); SHE, Syrian Hamster Embryo; TG, test guideline; TK, thymidine kinase; TTC, threshold of toxicological concern; UK NC3Rs, The National Centre for the Replacement, Refinement and Reduction of Animals in Research; WoE, weight of evidence.

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genotoxicity studies were used to further assess the relevance of positive *in vitro* findings for cosmetic ingredients. Due to the 7th amendment to the EU Cosmetics Directive testing ban (EU, 2003) that was enacted March 2009, it is no longer possible to conduct follow-up *in vivo* genotoxicity in this area. To address this, a workshop of COLIPA (The European Cosmetics Association) Safety Assessment and Genotoxicity project teams was held in Brussels on 3rd April 2008. Participants included members from a number of global cosmetic companies. The aim of the meeting was to (a) discuss company perspectives and current practices for the safety assessment of genotoxicity of cosmetic ingredients and (b) design a decision tree approach to the safety assessment of their potential to cause genotoxicity, with emphasis on non-animal methods. The outcome of this meeting is reported herein, starting with a review of the specific scientific challenges related to *in vitro* testing and the impact of the 7th Amendment. We describe research projects that have been undertaken by the COLIPA Genotoxicity Project Team to address the major limitations of the current *in vitro* testing paradigm. A proposed strategy is provided for the use of the non-animal methods to enable a thorough assessment of the genotoxic hazard of cosmetic ingredients.

2. Genotoxicity testing of cosmetic ingredients – challenges and approaches

2.1. In vitro genotoxicity testing leads to high percentage of misleading positive results

Due to the diverse nature of the mechanisms involved in genotoxicity, it is known that no single mutagenicity test can detect all classes and examples of genotoxic carcinogens. As a result, international guidelines for assessing the genotoxic potential of chemicals recommend the use of a battery of mutagenicity tests to detect gene, chromosome or genome mutations (Eastmond et al., 2009; Regulation, Evaluation, Authorisation of Chemicals (REACh); International Conference on Harmonisation (ICH), 2008; SCCP, 2006a,b, COM, 2000). For cosmetic ingredients, the Scientific Committee on Consumer Safety (SCCS, formerly the Scientific committee on Consumer Products (SCCP)) is the expert panel mandated by the European Commission to develop opinions for testing, review dossier submissions, and provide opinions concerning all types of safety risks. The SCCS recommended basic test battery for testing cosmetic ingredients for their genotoxic potential is: (1) two tests for gene mutation, the bacterial reverse mutation or "Ames" test (Organisation for Economic Co-operation and Development Test Guidelines (OECD TG) 471, 1997) and an *in vitro* gene mutation assay in mammalian cells (OECD TG 476, 1997) and (2) a test for clastogenicity and aneugenicity using the *in vitro* micronucleus test (MNT) (OECD TG 487, in development) (SCCP, 2006a,b). If all these tests are negative then no further testing is required.

The sensitivity and specificity of a number of *in vitro* genotoxicity assays in terms of predicting rodent carcinogenicity are shown in Table 1. Kirkland et al. (2005a, 2006) evaluated the predictivity of four standard *in vitro* tests for rodent carcinogenicity. The sensitivity of the MNT (i.e. ability to give positive results with rodent carcinogens) was the highest of the four individual tests analysed (though the database was smaller than the other assays) and the addition of the Ames assay increased the sensitivity further. For example, the sensitivity of the Ames assay was increased from 58.8% to 85.9% and 75.3% when it was combined with the mouse lymphoma assay (MLA), MNT or CA assay, respectively. Unfortunately, the specificity (the ability to correctly identify non-carcinogens) is greatly decreased with the addition of *in vitro* tests in a battery. For instance, the specificity of the Ames assay ranges from 74% to 80% (Table 1) and combining the Ames test with two other tests as required in the SCCS battery decreases the specificity to a low as 5–23% (Table 1). It is important to note that for some chem-

Table 1
A comparison of the sensitivity and specificity of some of the *in vitro* genotoxicity assays currently available. Taken from Kirkland et al., 2005b.

Test	OECD TG	Sensitivity to rodent carcinogens (%)	Specificity to rodent carcinogens (%)	Reference
Bacterial reverse mutation test, Ames test	471[a]	58.8	73.9	Kirkland et al. (2005a)
		45		Tennant et al. (1987)
		54		Zeiger (1998)
		49.4	80.3	Matthews et al. (2006)
In vitro micronucleus test (MNT)	Draft 487[b]	78.7	30.8	Kirkland et al. (2005a)
		87.3	23.1	Matthews et al. (2006)
		89.2	55.0	Corvi et al. (2008)
In vitro mammalian cell gene mutation test Mouse lymphoma assay (MLA) and hypoxanthine–guanine phosphoribosyl transferase (HPRT) test	476[c]	MLA/TK:	MLA/TK:	
		73.1	39.0	Kirkland et al. (2005a)
		70.9	57.8	Zeiger (1998)
		62.8, 70.9	44.2	Matthews et al. (2006)
		HPRT/CHO:		
		48.4	65.2	Matthews et al., 2006
In vitro mammalian chromosomal aberration assay	473[d]	65.6	44.9	Kirkland et al. (2005a)
		55.3	63.3	Matthews et al. (2006)
Ames + MNT		85.9	121.0	Kirkland et al. (2005a)
Ames + CA		75.3	34.6	Kirkland et al. (2005a)
Ames + MLA + MNT		90.7	5.0	Kirkland et al. (2005a)
Ames + MLA + CA		84.7	22.9	Kirkland et al. (2005a)
In vitro Syrian hamster embryo (SHE) cell transformation assay	Draft 495[e]	87	83	LeBoeuf et al. (1996) (24 h and 7 day)
		66	85	OECD DRP (pH 6.7; 7 day only)
		92	66	OECD DRP (pH 7.0; 7 day only)

[a] OECD TG 471 (1997).
[b] OECD TG 487 (2009).
[c] OECD TG 476 (1997).
[d] OECD TG 473 (1997).
[e] OECD TG 495.