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THINKING AHEAD IN TOXICOLOGY

SAFETY AND RISK ASSESSMENT OF PEPTIDES IN COSMETIC PRODUCTS

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Introduction

Recently, there has been increased interest in launching cosmetic products containing peptides to prevent or reduce skin aging in skincare cosmetics. The peptides can be extracted from several sources including plants, animals, or microorganisms, or can be produced synthetically.

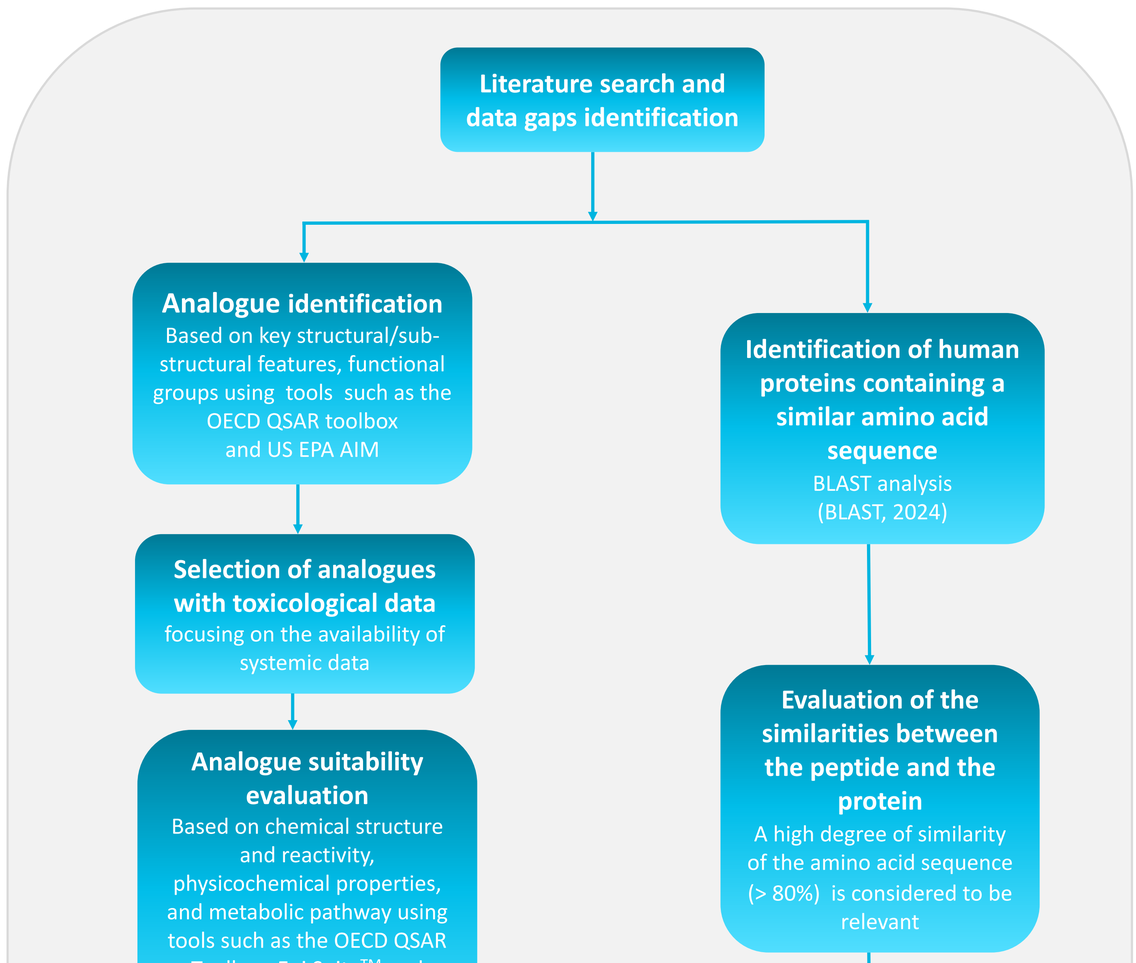
Peptides may be biologically active and have the potential to interact with skin. Thus, in line with the requirements stipulated under the EU Cosmetics Product Regulation ((EC) No 1223/2009) (EU, 2009), their safety must be established by selecting an appropriate Point of Departure (PoD) for systemic toxicity and a sufficiently high Margin of Safety (MoS) under cosmetic product use conditions.

This poster presents a weight-of-evidence approach to assess the safety of cosmetic products containing peptides, primarily based on the use of read across and BLAST (Basic Local Alignment Search tool) (NCBI, 2024).

In the context of read across, we have established a step-wise process to identify analogues and determine their suitability, in accordance with OECD guidelines and the ECHA read across assessment framework (RAAF) (ECHA, 2017). To increase confidence in the safety assessment, an additional analysis is conducted to determine whether the peptide of interest presents sequence similarity to known human proteins. Specifically, a BLAST analysis is performed to compare the peptide sequence to the National Center for Biotechnology Information (NCBI) database and identify the best similarity matches. A high degree of similarity (greater than 80%) with a known human protein can help assess the toxicological profile of the peptide. The safety assessment may be supported by results of *in vitro* testing to demonstrate hydrolysis and complemented with toxicological data on individual amino acids.

A case study is presented to demonstrate the safety assessment approach for data-poor peptides in cosmetics products.

Workflow for the safety assessment of peptides in cosmetics

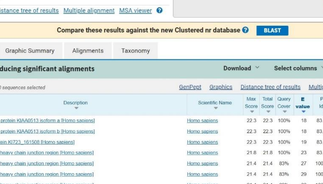


Case study : Data-poor peptide

OBJECTIVE

To establish a PoD and a sufficient MoS for peptide A, which is used to prevent or reduce skin aging in skincare cosmetics, using the systemic toxicity data available for an 'analogue'.

Figure 1: Peptide A

Steps	Available information
Literature search and data gaps identification	Available *in vitro* experimental data on the peptide A: skin and eye irritation, skin sensitisation, Ames test, and phototoxicity. Data gaps: acute toxicity, repeated dose toxicity, clastogenicity, carcinogenicity, reproductive, and developmental toxicity.
Analogue identification and evaluation	**Chemical name:** Bacitracin **Structure:**  (Yuan *et al.*, 2018) Analogue evaluation: • The analogue presents an acceptable similarity index (Dice index > 0.54). In addition, when comparing the amino acid composition, bacitracin shares three amino acids with peptide A. • The analogue shares key functional groups with peptide A. Bacitracin also contains additional functional groups due to the presence of different amino acids. • The (Q)SAR analysis using the OECD QSAR Toolbox v.4.6 showed that the analogue presents the same structural alerts for systemic toxicity, genotoxicity, carcinogenicity, and repeated dose. Bacitracin does not present an alert for eye irritation, while it presents an additional alert related to sensitisation. • Bacitracin presents physicochemical properties in the same range compared to the target substance. • Finally, *in vivo* metabolism data is available for bacitracin, which has been shown to undergo hydrolysis via peptidases as first metabolic reaction (EMEA, 1998). The target substance is also predicted by Meteor® Nexus (Judson *et al.*, 2015) v.3.2.0 to undergo hydrolysis as first metabolic reaction.
Identification of similar human peptides to support repeated dose toxicity data of the analogue	BLAST analysis identified a known human protein with a high degree of similarity. In fact, the target substance shows 83% sequence similarity with the human immunoglobulin heavy chain junction region, corresponding to a part of the antigen recognition site for the antigen – antibody interaction. **Figure 2:** Blast analysis
Safety assessment based on a weight-of-evidence approach	• The skin and eye irritation, skin sensitisation, Ames test, and phototoxicity endpoints were addressed using *in vitro* data on peptide A. • The acute toxicity, repeated dose toxicity, clastogenicity, carcinogenicity, reproductive and developmental toxicity endpoints were addressed using the *in vivo* data available for the analogue bacitracin. • BLAST analysis confirmed a similarity with a region of an endogenous human protein • The systemic toxicity was further supported by the safety data on the individual amino acids, as an *in vitro* study using hepatocytes supported a high rate of metabolism and clearance of the peptide A.
Determination of the PoD and MoS calculations	• Based on the available studies, a LOAEL of 11 mg/kg bw/day derived from a 13-week rat study available for bacitracin was established as the most relevant PoD for the safety assessment of the peptide A. • A comparison of the systemic exposure dose of peptide A with the PoD resulted in an MoS of >1000, which is well above the threshold of 100 for concluding safe use.

Conclusion

Based on 1) *in vitro* experimental data, 2) *in vivo* data on the suitable analogue, 3) supporting data from the Blast analysis and individual amino acids, an appropriate PoD for systemic toxicity and a sufficiently high MoS can be established under the use condition of the cosmetic product.

References

CIR, 2012. Safety Assessment of α-Amino Acids as Used in Cosmetics, Final Report for Public Distribution. Cosmetic Ingredient Review, https://www.cir-safety.org/sites/default/files/amacid092012rep.pdf.

ECHA, 2017. Read-across assessment framework (RAAF). https://echa.europa.eu/documents/10162/13628/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a

NCBI, 2024. BLAST ® » blastp suite. https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins.

EMEA. 1998. Committee for veterinary medical products: Bacitracin - summary report. Report No. EMEA/MRL/451/98-FINAL, https://www.ema.europa.eu/en/documents/mrl-report/bacitracin-summary-report-committee-veterinary-medicinal-products_en.pdf.

EU, 2009. Regulation (EC) No 1223/2009 of the European parliament and of the council of 30 November 2009 on cosmetic products. L 342/59. Official Journal of the European Union, https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1223&from=EN.

JECFA, 2006. Amino acids and related substances, safety evaluation of certain food additives and contaminants, sixty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Report No. 54, http://whqlibdoc.who.int/publications/2006/9241660546_eng.pdf

Judson PN *et al.,* 2015. Assessing confidence in predictions using veracity and utility – A case study on the prediction of mammalian metabolism by Meteor Nexus. Mol Inform 34 (5):284-291.

Yuan K, *et al*. 2018. Antimicrobial peptide based magnetic recognition elements and Au@Ag-GO SERS tags with stable internal standards: a three in one biosensor for isolation, discrimination and killing of multiple bacteria in whole blood. Chem Sci 9 (47):8781-8795.

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