Next generation risk assessment of hair dye HC yellow no. 13: Ensuring protection from liver steatogenic effects

Commonly used acronym: *NGRA*

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Organisation

Name of the organisation Vrije Universiteit Brussel (VUB)

Specific Research Group or Service

In Vitro Toxicology and Dermato-Cosmetology (IVTD)

Country Belgium

Geographical Area Brussels Region

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Regulatory use - Routine production
Type of method	Other: Integrated NGRA approach combining in vitro bioassays, QSAR modelling, and PBK-based exposure modelling for liver steatosis risk assessment

DESCRIPTION

Method keywords

NGRA

new approach methodologies

in vitro

in silico

PBK modelling

QSAR

read-across

liver steatosis

cosmetic safety assessment

hair dye

Scientific area keywords

Toxicology

risk assessment

computational modelling

Regulatory Science

Systems Toxicology

Chemical Safety

Mechanistic toxicology

Cosmetic regulation

Method description

This Next Generation Risk Assessment (NGRA) approach integrates *in vitro* bioactivity data, *in silico* modelling, and exposure-based modelling to assess the liver steatosis risk of the cosmetic hair dye

ingredient HC Yellow No. 13. The workflow follows the principles of animal-free, exposure-led safety assessment and combines mechanistic data from human cell models with computational tools, including QSAR analysis, read-across, and physiologically based kinetic (PBK) modelling. Together, these components establish a weight-of-evidence evaluation to ensure that bioactivity levels observed *in vitro* are below those expected from consumer exposure. The approach demonstrates how integrated New Approach Methodologies (NAMs) can support regulatory decision-making under the EU Cosmetics Regulation without reliance on animal testing.

Lab equipment

- Standard cell culture facilities (biosafety cabinet, CO? incubator, microscope, centrifuge, and plate reader) for maintaining and treating human stem cell–derived hepatic cultures (hSKP-HPC).

- Analytical instrumentation includes a flow cytometer for semi-quantitative lipid accumulation analysis, and a spectrophotometric plate reader for colorimetric triglyceride assays.

- Computational tools include QSAR software, PBPK modelling platforms (e.g., GastroPlus), and data analysis software (e.g., R, PROAST).

Method status

History of use

Internally validated

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- This NGRA integrates multiple New Approach Methodologies (NAMs), including *in vitro* hepatocellular assays, *in silico* modelling, and PBK-based exposure estimation, to provide a fully animal-free, exposure-led safety assessment.

- It demonstrates how a mechanistic and tiered weight-of-evidence approach can ensure consumer protection while aligning with EU regulatory requirements.

- The method allows identification of liver steatosis potential at human-relevant concentrations, improves mechanistic transparency through AOP-based reasoning, and reduces uncertainty by combining diverse data streams.

Challenges

- The workflow's main limitation lies in the current lack of validated and harmonised *in vitro* assays for repeated-dose liver toxicity.

- Quantitative integration of *in vitro* bioactivity with PBK-derived internal concentrations still relies on assumptions about metabolic competence and the relevance of chronic exposure.

- Data curation and interoperability between computational and experimental layers remain technically demanding, and regulatory acceptance of integrated NGRA frameworks is still evolving.

Modifications

- Ongoing work focuses on extending the approach to include omics-based mechanistic profiling, sensitivity analysis of PBK parameters, and integration with knowledge graph tools, such as TOXIN-KG, to support read-across justification.

- Future refinements will focus on improving the linkage between *in vitro* kinetics, exposure duration, and apical outcomes to strengthen quantitative risk characterisation.

- Cross-laboratory testing could enhance reproducibility and reliability for regulatory use.

Future & Other applications

- The method can be adapted for other cosmetic ingredients or industrial chemicals where hepatotoxicity, steatosis, or metabolic disruption are relevant endpoints.

- It is also applicable to broader NGRA case studies for repeated-dose systemic toxicity, helping to establish practical workflows for safety substantiation under the EU Cosmetics Regulation and REACH.

- With further optimisation, the framework could contribute to next-generation risk assessments across different organs and product sectors.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Sepehri, S., De Win, D., Heymans, A., Van Goethem, F., Rodrigues, R. M., Rogiers, V., & Vanhaecke, T. (2025). Next generation risk assessment of hair dye HC yellow no. 13: Ensuring protection from liver steatogenic effects. Regulatory Toxicology and Pharmacology, 159, 105794. https://doi.org/10.1016/j.yrtph.2025.105794

Associated documents

1-s2.0-S0273230025000248-main (2).pdf

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