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Ab initio chemical safety assessment: A workflow based on exposure considerations and non-animal methods

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ABSTRACT

We describe and illustrate a workflow for chemical safety assessment that completely avoids animal testing. The workflow, which was developed within the SEURAT-1 initiative, is designed to be applicable to cosmetic ingredients as well as to other types of chemicals, e.g. active ingredients in plant protection products, biocides or pharmaceuticals. The aim of this work was to develop a workflow to assess chemical safety without relying on any animal testing, but instead constructing a hypothesis based on existing data, *in silico* modelling, biokinetic considerations and then by targeted non-animal testing. For illustrative purposes, we consider a hypothetical new ingredient x as a new component in a body lotion formulation. The workflow is divided into tiers in which points of departure are established through *in vitro* testing and *in silico* prediction, as the basis for estimating a safe external dose in a repeated use scenario. The workflow includes a series of possible exit (decision) points, with increasing levels of confidence, based on the sequential application of the Threshold of Toxicological (TTC) approach, read-across, followed by an "ab initio" assessment, in which chemical safety is determined entirely by new *in vitro* testing and *in vitro* to *in vivo* extrapolation by means of mathematical modelling. We believe that this workflow could be applied as a tool to inform targeted and toxicologically relevant *in vitro* testing, where necessary, and to gain confidence in safety decision making without the need for animal testing.

Introduction

Within the European Union (EU) innovations in the safety assessment of chemicals are required to support the EU policy to protect laboratory animals [30] and to provide new regulatory acceptable assessment approaches, especially after the full implementation of the EU Cosmetics Regulation [18]. Therefore the European Commission, within the frame of the FP7 Health Programme (https://ec.europa.eu/research/fp7/), together with Cosmetics Europe (https://www.cosmeticseurope.eu/) co-financed the research initiative "Safety Evaluation Ultimately Replacing Animal Testing (SEURAT)" (http://www.seurat-1.eu) in a public–private partnership [35]. The initiative was strongly inspired by the U.S. National Research Council report entitled *Toxicity Testing in the 21st century: A Vision and a Strategy* [68]. SEURAT-1 was planned to be a first step to address the long term strategic target, focusing on the replacement of animal testing in chemical assessment for repeated dose systemic toxicity. Six research projects and a coordination action contributed to the initiative, and combined the research efforts of over 70 European universities, public research institutes, and companies. The SEURAT-1 strategy [94] adopts a toxicological mode-of-action MoA) framework to describe how any substance may adversely affect human health [3,8,56] and uses this knowledge to develop complementary theoretical, computational (*in silico*), and experimental (*in vitro*) models that enable prediction of quantitative points of departure, needed for safety assessments [84]. The research initiative aimed to prove this concept on three levels

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[94,95]: (1) theoretical descriptions of adverse outcome pathways (AOP) based on existing knowledge, (2) toxicity prediction based on hypothesis-driven testing employing *in vitro* and *in silico* methods, and (3) safety assessment applying existing information strengthened with selected data generated from alternative methods suitable for regulatory use.

SEURAT-1 undertook the *"ab initio"* case study by applying SEURAT-1 methods and approaches, as well as results from already existing alternative testing, e.g. ToxCast [29]. The aim was to develop a structured risk assessment workflow for repeated dose toxicity, with the goal of predicting a no-adverse effect level of a cosmetic relevant ingredient, assuming a certain exposure scenario. Within the context of this workflow, the Threshold of Toxicological Concern (TTC) approach, evaluated by the COSMOS project (http://www.cosmostox.eu/; [98,99]) and refined for dermal exposure [97], was applied to support a low exposure scenario. In addition, read-across was incorporated to strengthen the non-animal evidence with structurally similar substances and make biological links to higher order outcomes [3,8]. The application of the TTC approach and read-across was followed by a so-called "ab initio assessment", meaning that the safety evaluation was carried out on the basis of hypothesis-driven *in vitro* testing combined with *in vitro* to *in vivo* extrapolation by computational modelling. While it was not considered realistic to fully complete such a risk assessment for a chosen substance within SEURAT-1, the case study is the basis for an integrated assessment that relies only on alternative methods. It showcases the feasibility of carrying out such an assessment, but also illustrates uncertainties and knowledge gaps. These learnings will assist in shaping a more focused strategy to advance alternative safety assessment approaches.

Within SEURAT-1, a conceptual framework for safety assessment was developed [13] outlining a logical basis for the different steps in a chemical safety assessment without performing additional animal testing. The conceptual framework was intended to provide the basis for the feasible design of integrated assessment approaches which can be adapted for a particular case depending on the purpose of the prediction, and the degree of uncertainty that can be tolerated. The overall outcome of an assessment based on the framework is anticipated to be robust as it is based on multiple pieces of evidence. Nevertheless the type and degree of uncertainty in the predictions needs to be understood to ensure that the assessment is 'fit for purpose'.

The framework takes into account whether the substance is likely to exhibit general toxicity or a specific biological MoA. A large number of substances are assumed to provoke general toxicity [88], i.e. they tend to be 'unselective' in interacting with biological targets and hence have the potential for generic biological perturbation. Other substances, for example often in the case of pharmaceuticals or pesticides, are 'selective' in interacting with biological targets and have a known biological mechanism. Information on toxicokinetics and toxicodynamics are important in either case.

The safety assessment workflow developed here is based on the general SEURAT-1 conceptual framework. As stated earlier, applying the framework to an *ab initio* assessment at this point in time was a stretch goal aiming to highlight gaps for future development and illustrate overall progress made in SEURAT-1. It assists in structuring the information and provides guidance regarding what additional alternative data are needed to establish and then test a hypothesis. The assessment is based on gathering existing data and using information from alternative methods, as described in the guidelines for safety evaluation of cosmetic substances, which were developed and are regularly updated by the Scientific Committee on Consumer Safety [78]. We are here going further by organising the information into a logic workflow and by starting with exposure considerations so that both hazard and risk are incorporated into the *ab initio* assessment. Moreover our intention is that the workflow is general enough to cover any type of chemical and exposure, and need not be limited to cosmetics. The chemical to be assessed in the *ab initio* workflow can be a substance synthesized or extracted from natural source for the very first time or an existing challenged ingredient. The workflow could also be applicable to an already manufactured substance with a new intended use resulting in higher exposures that extends beyond previous assessments. The workflow starts from the same considerations regardless of the type of safety assessment. The starting point is Tier 0 where the exposure scenario and chemical identity are defined. This initial tier includes exit points where the TTC approach or a read-across assessment based on chemical similarity could be applied. In cases where neither of these approaches is considered to be adequate, it is necessary to proceed with applying the workflow. In the following steps, high throughput or high content data from alternative methods are collected under Tier 1 to better understand possible MoA, while Tier 2 is targeted testing based on the hypothesis(es) set up under Tier 1.

To illustrate this workflow a case study with a hypothetical exposure scenario was created for the substance x: a new ingredient introduced in a body lotion formulation, which is applied twice per day on skin (overall body surface).

A workflow for chemical safety assessment with non-animal methods

We here outline a general workflow for chemical safety assessment (Fig. 1), based on the SEURAT-1 conceptual framework, but further elaborated, aiming to provide an tool to guide the assessor through the different steps to be considered and enable decision making. *Ab initio* means 'from the beginning'; in the context of this workflow, *ab initio* assessment refers to the hypothesis-driven generation of new *in vitro* data and data interpretation (*in vitro* to *in vivo* extrapolation) by means of mathematical modelling. The more robust the information we manage to collect, the better it can assist us in making the hypothesis, and the better we are guided in identifying data gaps and elaborating a targeted testing strategy with a call for data as needed. We use existing human and animal data, when available, to underpin a hypothesis in combination with existing and generated *in silico* and *in vitro* data to provide the basis for targeted testing applying selected alternative methods. To provide confidence in the assessment, the level of uncertainty must be estimated for each step. If the uncertainty at the end is too large, the assessment will not be useful as such, but will be the basis for identifying the remaining gaps that are likely caused by lack of relevant and reliable methods. The workflow allows us to apply Thresholds of Toxicological Concern (TTC) or read-across approaches. These are indicated but not detailed further here as these approaches were described in other SEURAT-1 safety assessment case studies [6,80,97]. These approaches are treated as "exits" in the *ab initio* workflow, and it should be noted that they have already reached a certain degree of regulatory acceptance [19–21,22,76,77].

The general workflow is illustrated in Fig. 1, and is step-wise described here below.

TIER 0: Identify use scenario, chemical of interest and collect existing information.

Identify exposure/use scenario

If the chemical is part of a product and chemical release from the product matrix can be excluded, the chemical can be safely used because there is no exposure to the chemical from the product, e.g. exposure-based waiving under REACH [17] is applied. Of course it must be carefully evaluated whether there are any additional uses to be considered in the assessment when the chemical is or can become available during the lifecycle of the product (e.g. production and waste treatment of the product).

When describing the exposure scenario, it should be considered whether the exposure is intentional or not, and in both cases estimates of dose, expected routes of exposure, frequency and length of exposure, should be made. It might also be relevant to consider more than one