Use of New Approach Methodologies for the hazard assessment of nanocellulose oral exposure: the EFSA granted NANOCELLUP project

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INTRODUCTION

Nanocellulose (NC) is an emerging material in the food sector with foreseen applications in food packaging, as novel food and food additive. However the potential hazards of ingested NC are insufficiently characterised. The nanoscale features of NC require a nano-specific assessment (EFSA SC, 2021), which should focus on NC's potential to elicit local adverse effects in the gut and/or to cross the intestinal barrier, leading to systemic exposure. The possible degradation of NC by the human microbiome, potentially delivering smaller fibres in the large intestine, is another open question. Laboratory animals do not seem appropriate models because their digestive physiology, microbiome and rate of fibre degradation differs from humans.

NANOCELLUP (GP/EFSA/SCER/2020/03/4/Lot1) aims to (i) design and conduct a set of NAM-based studies for addressing the current knowledge gaps on NC hazards and (ii) offer a proposal for including the results in the regulatory hazard assessment of NC dietary exposure. A battery of in vitro tests will provide insight into NC hazard(s) and mode(s) of action and will assess any relationship between toxicity and physicochemical characteristics.

METHODOLOGY

Mono- and co-culture systems will be used and specific endpoints will be considered to investigate potential effects of NC such as impaired cell viability/cytotoxicity, oxidative stress responses, (pro-)inflammatory responses and integrity of the intestinal barrier. A tiered approach will be followed. The objective of the Tier 1 studies will be to obtain a maximum amount of information on the cellular responses following exposure to a panel of NC samples. Results from Tier 1 studies will be used for the final selection of the NC (2-3 materials) for further investigation in Tier 2, which will focus on:

1. Digestion or degradation, including surface modifications, of NC by the human microbiome;
2. Assessment of the uptake and crossing of the intestinal barrier by NC;
3. Assessment of local effects, including inflammation and genotoxicity, of NC on the intestinal epithelium.

The material showing the most remarkable effects in Tier 2 testing (or in absence of effects the material with physicochemical properties likely associated with increased hazard or showing some degree of uptake/translocation) will be submitted to Tier 3 testing, in which repeated dose toxicity will be investigated.

RESULTS

The first two milestones of the project are being attained. The first is the selection of a wide panel of NC samples to be submitted to Tier 1 testing. Three NC types exist: (i) materials produced by bacterial species (BNC) and other NCs obtained by technological modification of cellulose from plants or other origins, leading to (ii) nanofibrillated cellulose (NFC) or (iii) cellulose nanocrystals (CNC). The biological sources and preparation conditions have been shown to affect several physicochemical parameters of NC. In order to capture this variability as much as possible up to ten samples are being selected with CNC predominating, followed by NFC and BNC.

The second milestone is the optimisation of electron microscopy-based approaches for the physicochemical characterisation of pristine NC and in situ visualisation of fibres (in cells and in vitro colonic samples). For method validation, CNC certified reference material has been used. Detection in colonic material will be checked by preliminary experiments in a batch fermentation system. Afterwards, NC degradation by human microbiome will be studied by means of a validated, dynamic in vitro model of the human colon (ARCOL).

DISCUSSION

The increasing use of NC in food applications requires an assessment according to the Guidance for Risk Assessment of Nanomaterials (EFSA SC, 2021). One main concern is that the nanoscale features of the material may influence its toxicokinetic behaviour (i.e. Are the nanosized fibres able to cross the intestinal epithelium? Is their uptake driven by their physicochemical properties, leading to selective uptake of nanofibers with specific characteristics?). Another concern is represented by the unknown hazardous properties of cellulose nanofibers and the possible relationship with characteristics of the material (e.g. length/shape of the fibres). In order to address these concerns, a battery of in vitro tests providing insight into NC hazard and mode of action are used in NANOCELLUP. Intestinal uptake of NC is being studied using single cell lines of enterocytes (differentiated Caco-2) and macrophages (THP1), whereas for investigating uptake and crossing of the intestinal barrier a tri-culture of Caco-2, HT29 and Raji B cells is being used. Experiments are designed so as to ensure relevance and reliability of the results in the perspective of regulatory risk assessment.