As part of the internal Safety Project, the reprotoxicity/teratogenicity pathway is a high priority research axis. The overall objective is the development and the evaluation of alternative methods aiming at an ITS (Integrated tested Strategy) and a Teratogen Index tools for reprotoxicity evaluation of Raw Materials.

In a first step, 85 compounds (45 negative and 40 positive) were selected from public databases (e.g., ECHA, LOLI, DART) as training set, to be screened for their reproductive and teratogenic potential through different methods developed along one objective: Development or implementation of established methods based on emerging topics in human toxicology impact assessment.

Three developed tests are being used to assess the teratogenic potential: i. A test on human Induced Pluripotent Stem cell (devTOX quickPredict™) ii. Two tests on Fish Embryo models: Zebrafish (Biobide) and Medaka iii. In Silico prediction as weight of evidence for biological coverage for complementarity with testing (endpoint gap, performance, …) and/or improve testing results (in silico physchem, metabolism, …).

Other innovative methods are being characterized (i.e; Placental Barrier Model, Zebrafish Embryo Metabolization, Toxicogenomics…) and could be integrated in the ITS.

In conclusion, the Biobide, Stemina and DART complementation intermediate analysis on 41 compounds showed good sensitivity and specificity and appears to be an interesting predictive approach. The addition of progressive substances in the tests battery as well as the implementation of new methods would allow to refine the results to obtain a robust ITS and to evaluate the teratogenic potential of the substances.

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Development of novel nanotoxicity assessment method utilizing 3D printing system

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Unique physicochemical properties of nanomaterials (NMs) make them a material of choice in various applications but also raise concerns about their potential toxicity. While the commercial use of nano-enabled materials is growing rapidly, their interaction with biological systems and environment are not yet fully understood [1, 2]. Traditionally, toxicity of nano-sized materials are assessed by 2D cell culture models due to their time and cost-related advantages but their simplicity often comes at the cost of accuracy. While these methods are considered as the first step in toxicological assessment of both nano-sized and bulk-form materials, they fall short in mimicking the complexity of *in vivo* physiological environments. The static nature of 2D models result in the loss of dynamic cell-cell and cell-environment interactions that play critical roles in key mechanisms such as cell differentiation, proliferation, vitality, responsiveness to stimuli and drug metabolism, and subsequently, result in the loss of diverse phenotypes. Moreover, 2D cell culture designs provide unlimited access to nutrients, metabolites and signaling molecules unlike *in vivo* systems [3–5]. Taken together, the above-mentioned disadvantages of 2D cell culture models necessitate the development of 3D cell culture methods for accurate and realistic toxicological evaluation of NMs.

Here, we propose a novel nanotoxicology screening method that is based on a 3D printed scaffold from soybean oil epoxidized acrylate, combined with a flow system to overcome limited mass transport in static cell culture systems. Soybean oil epoxidized acrylate was selected as resin for fabricating scaffold via SLA 3D printing due to its cost effective, environment friendly, and biocompatible nature. The system was composed of an *in house* built precise peristaltic pump, which fed media to the Quasi Vivo ® chamber containing the 3D printed scaffold. Photon Mono X 3D printer (Anycubic 3D Printing, China) was used to produce 3D scaffolds. The CAD software Fusion 360 (Autodesk Inc., USA) was used to design the 3 × 3 × 5 mm (W × D×H) scaffolds of 100 µm thick fibers with 100 µm spaces, rotating 90 degrees every layer. The parameters of printing process were adjusted to 50 µm layer height and 1.6 seconds exposure per layer. Precise peristaltic pump was built from Arduino controlled step motor and 3D printed casing. The 3D printed scaffold structure was inserted into Quasi Vivo ® chamber. Initial case studies confirmed that the system was running smoothly and it could potentially be used for nanotoxicological assessments. Next step is to demonstrate the application of this novel 3D cell culture system to the toxicological evaluation of metal oxide NMs against human liver cells. Once fully optimized, the developed system will provide an alternative, cheap and robust way of assessing the toxicity of ever-expanding NMs.

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P17-22
Zebrafish, a novel key player for human risk assessment: latest advances on developmental neurotoxicity from an international consortium

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