Report on the proof-of-concept evaluation of SOPs for innovative *in vitro* models and mechanistically relevant assays for nanosafety human hazard assessment

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Background

- **Task 5.3** of WP5 (Human Hazard Assessment) led by Swansea University (SU) was focused on the evaluation of advanced *in vitro* models, as compared to standard *in vitro* and *in vivo* hazard characterisation systems for engineered nanomaterials (ENMs).
- The aim of this task was to compare the ability of advanced model systems cultured either in three dimensions (3D) or of multiple cell types to report on hazard endpoints when exposed to ENMs.
- Their performance has been compared to the equivalent outputs from less complex, yet well-established 2D monoculture systems using an extensive systematic literature approach.
- The combination of published literature and the new data generated in the Task allowed us to determine if the innovative advanced models are more-, or less-predictive of nanomaterial-induced damage than monocultures.

Methods

- The first element consisted of a review of the literature following the GUIDEnano principles (critically appraising publications based on their scientific quality), focusing on the liver and lung – two key organs commonly used to model nanomaterial exposure.
- The review was broken down into three sections; advanced *in vitro* models, well-established 2D *in vitro* models and finally a comparison against *in vivo* models.
- The analysis directed our focus to the HepG2 liver spheroid model developed in the PATROLS project and a co-culture lung model developed by LIST.
- A small interlaboratory trial was established between to determine if reproducible data could be generated using known operating protocols for model.
- Cytotoxicity and (pro)-inflammatory response were selected for biological endpoints, nanomaterial exposures were conducted using the VitroCell (12-well) cloud system and in submerged media conditions for the HepG2 spheroids.

Results

- Figure 1 summarises the data generated using the LIST lung model consisting of A549, EA.hy926 and differentiated THP-1 (d.THP-1) cells.
- Nanomaterial deposition was consistent across both laboratories utilizing the VitroCell Cloud-12 system.
- No significant release of IL-8 was observed at both laboratories however laboratory 1 did report significant cytotoxicity for CuO which was not replicated in laboratory 2.

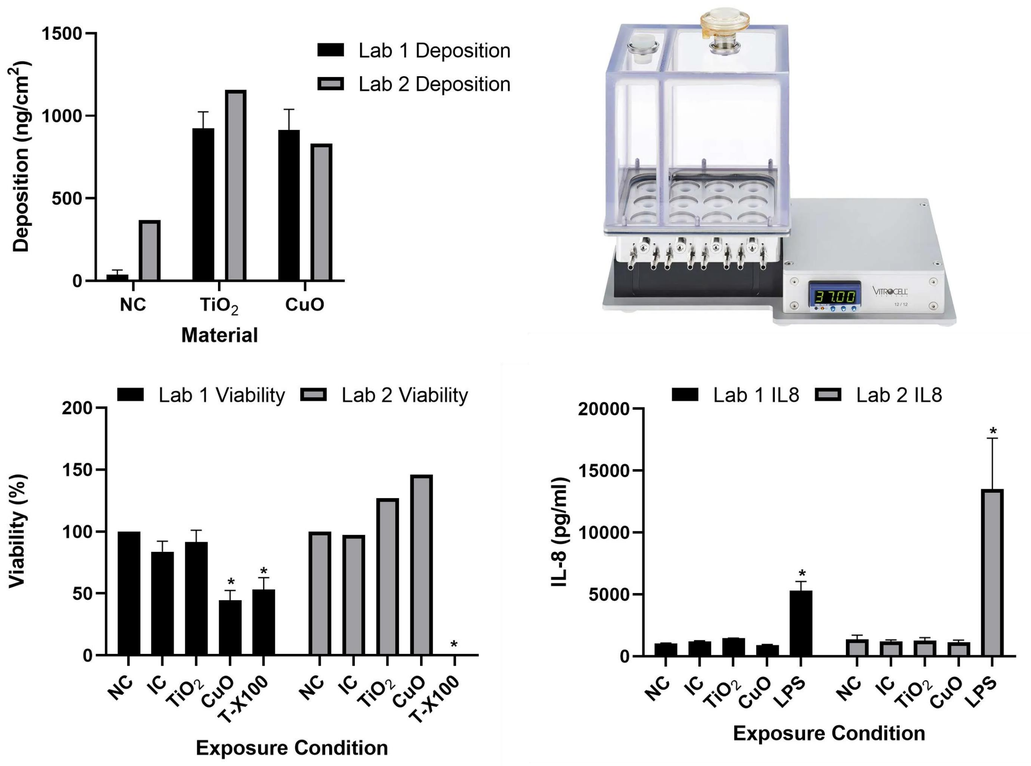

Figure 1. Nanomaterial deposition rates, cytotoxicity and (pro)-inflammatory response data generated through a small interlaboratory trial.

- Figure 2 summarises the data generated using the PATROLS HepG2 liver spheroid model.
- All contributing laboratories generated harmonised data for both biological endpoints of cell viability and (pro)-inflammatory response.
- Three laboratories participated in the interlaboratory trial, data displayed is for laboratories 1 and 2, final data set currently being finalised.

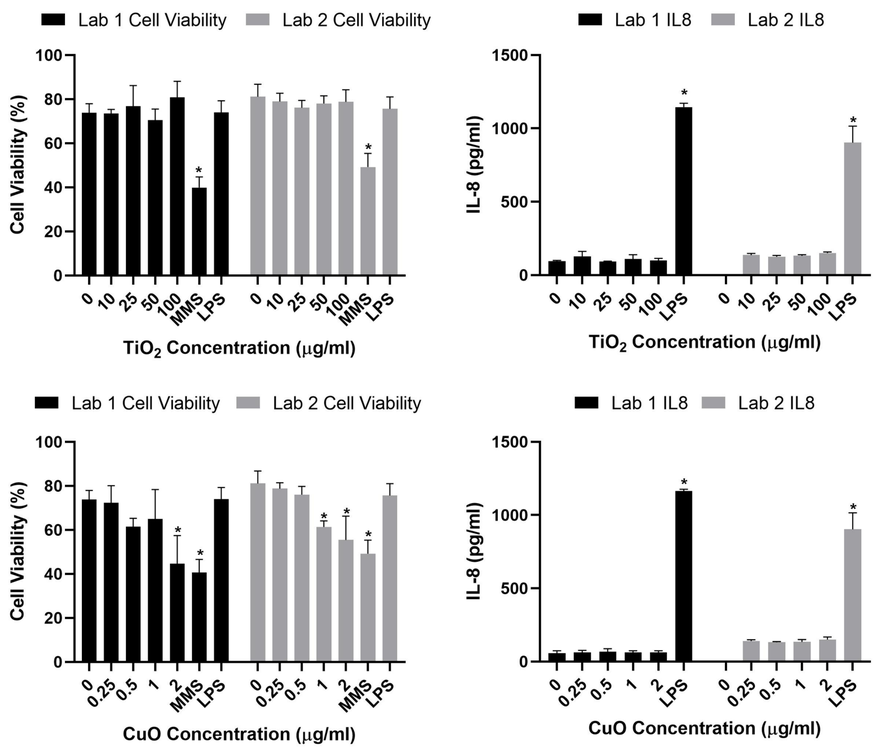

Figure 2. HepG2 Spheroid cytotoxicity and (pro)-inflammatory response data generated through a small interlaboratory trial.

Summary

- **LIVER:** The data generated in each laboratory concerning the HepG2 spheroid models showed a high degree of concordance for cell viability via TBE and (pro)-inflammatory response. Therefore, it can be concluded that each laboratory, whilst following the PATROLS protocol for acute ENM exposures to HepG2 spheroids cultured via the hanging drop method, produced a harmonised data set.
- **LUNG:** Differences were recorded between the two laboratories in terms of particle deposition efficiency. The negative control contribution was unexpectedly high, while TiO_2 and CuO deposition were close to the targeted dose of 1000 ng/cm^2.
- **LUNG:** No cytotoxicity (via Alamar Blue) was observed after exposure to TiO_2. Different responses were observed in laboratory 1 and 2 when CuO was administered.
- **LUNG:** Despite the differences observed by the two laboratories in cell viability, the quantification of IL8 after exposure to the ENMs was consistent.
- **LUNG:** At the concentrations applied, both the ENMs did not induce a significant release of IL8. The 3D models were responsive to LPS (1 mg/ml).

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