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Development of an "agitated" *in-vitro* test for glass fiber dissolution

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Mineral (glass and stone) wool is one of the most used insulation materials, due to its outstanding effectiveness, but also because of extensive and robust studies supporting the fact that they are safe to use when standard safe work practices are followed. Specific protocols to characterize fiber biopersistence have been defined by European authorities (Bernstein et al., 1999). Tested according to one of these protocols, the fibers have to demonstrate a low biopersistence to not be classified as carcinogenic under the EU CLP Regulation (Note Q, Regulation (EC) n°1272/2008). Mineral wool manufacturers, such as Saint-Gobain, need to reduce as much as possible the number of these *in-vivo* tests, as they raise ethical issues and are costly and time-consuming. Thus, the development of *in-vitro* tests reliable, quicker, cheaper and predictive of *in-vivo* biopersistence is required.

EURIMA (EURopean Insulation Manufacturers Association) (Sebastian et al., 2002) aims to develop an acellular *in-vitro* test, in which fibers dissolution in a flow-through system is followed by chemical analysis of the solutions. Saint-Gobain has a long experience on dissolution tests (de Meringo et al., 1994; Thelohan et al., 1994; Guldberg et al., 2000) and has decided to develop an acellular *in-vitro* test in a different way, with a closed system in which the dissolution fluid is agitated but not circulating.

In this work, the Saint-Gobain *in-vitro* test is presented; the parameters impacting glass wool and stone wool dissolution, such as the fluid composition and pH, are studied; and differences between "circulating" and "agitated" *in-vitro* dissolution tests are discussed. The "agitated" fluid allows to reduce the test duration and to maintain a constant pH during experiment, which increases the reproducibility. This test is still under development as it also displays limits. Further efforts will be needed to obtain a robust predictive tool.

References

Bernstein, D. and Sintes, R. (1999). Methods for the Determination of Hazardous Properties for Human Health of Man Made Mineral Fibres (MMMF). EUR 18748 EN.

de Meringo, A., Morscheidt, C., Thélohan, S. et al. (1994). In Vitro assessment of biodurability: Acellular systems. *Environ Health Perspect 102*, 47-53.

Guldberg, M., de Meringo, A., Kamstrup, O. et al. (2000). The development of glass and stone wool compositions with increased biosolubility. *Regul Toxicol Pharmacol 32*, 184-189.

Sebastian, K., Fellman, J., Potter, R. et al. (2002). EURIMA test guideline: In-vitro acellular dissolution of man-made vitreous silicate fibres. *Glass Sci Technol 75*, 263-270.

Thelohan, S. and de Meringo, A. (1994). In vitro dynamic solubility test: Influence of various parameters. *Environ Health Perspect 102*, 91-96.

Presentation: Posters

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IPAM, the Italian Platform on Alternative Methods

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National Platforms for alternative methods were created about twenty years ago with the aim to promote and inform about alternative methods to speed up their regulatory acceptance. To achieve these goals effectively and incisively, collaboration between the following four areas was considered as a priority: Research – Industry – Government Institutions – Associations for animal protection.

The Italian Platform on Alternative Methods (IPAM), in full compliance with this approach, pursues at: i) promoting research and information on alternative methods in animal experimentation in Italy, ii) building synergies to accelerate development and acceptance of alternatives methods in basic, applied, and regulatory research. Within this framework, IPAM regularly organizes national and international events and meetings (Rovida et al., 2013; Nagy et al., 2016; Caloni et al., 2018) aimed at different stakeholders (students, researchers, general public). Among the most recent, the exhibition "Science & Consciousness, a journey inside the 3Rs" a didactic itinerary on alternatives in animal experimentation which was hosted by several Italian universities and research institutes, and the IPAM-ecopa symposium 2019 on "Non Animal Methodologies (NAMs): research, testing, assessment and applications", recently co-organized with ecopa (European consensus-platform for alternatives) (Lorenzetti et al., 2020).

Moreover, since 2007, the IPAM-Farmindustria award is assigned every two years to a young researcher author of a paper and/or a thesis degree relevant to the application of 3Rs Principle in pharmacological research. IPAM also actively dialogue with national and international regulatory bodies and its members frequently share their expertise in training events organized by university, industries, and public entities.

Finally, the IPAM's website (www.ipamitalia.org) and Facebook social (www.facebook.com/IPAMITALIA) represent an important point of reference of information, updates, and discussion for anyone who is interested on the 3R Principle, alternative methods and their applications in science.

References

Caloni, F., Sambuy, Y., Lombardi, G. et al. (2018). 3Replacement winter school – Out of the barriers: In vitro models in toxicology. *ALTEX 35*, 520-521. doi:10.14573/altex.1807231

Lorenzetti, S., Aquilina, G., Caloni, F. et al. (2020). Non animal methodologies (NAMs): Research, testing, assessment and applications – ecopa symposium 2019. *ALTEX 37*, 317-320. doi:10.14573/altex.2003041

Nagy, A. L., Oros, N. A., Farca, L. et al. (2016). 3Rs in education. *ALTEX 33*, 185-186. doi:10.14573/altex.1602011

Rovida, C., De Angelis, I. and Lorenzetti, S. (2013). Alternative in vitro methods to characterize the role of endocrine active substances (EASs) in hormone-targeted tissues. *ALTEX 30*, 253-255. doi:10.14573/altex.2013.2.253

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A novel *in silico* tool for dose assessment in cell monolayer nanotoxicology

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Due to the widespread use of Engineered Nanomaterials (ENMs), *in vitro* ENM dose assessment appears crucial in nanotoxicology (Sohal et al., 2018). In this context, *in silico* modelling provides an outstanding tool for predicting the amount of ENMs delivered to biological tissues in specific configurations (Poli et al., 2020). In literature, two computational models have emerged regarding ENM dosimetry: the DG (DeLoid et al., 2015) and the ISD3 model (Thomas et al., 2018), implementing the 1D dynamics of nanoparticles through a protein-enriched culture medium with a cell monolayer on the bottom.

In this study, we firstly integrated these models within a Graphical User Interface (GUI) purposely developed in Matlab, enabling to identify the most suitable one for a specific application. Then, we exploited it for insoluble ENM (i.e., CeO_2, TiO_2, and $BaSO_4$) dosimetry. The predictions of the GUI in terms of middle height concentration profiles over time for such ENMs were successfully validated ($R2 > 0.75$) by fitting them on the corresponding experimental profiles, measured using a gelatin coating for mimicking the totally sticky bottom condition implemented *in silico*.

Moreover, since nanoparticle uptake by cells (i.e., the bottom stickiness) is an ENM-specific feature, we exploited a plasma-spectrometer for assessing the cumulative dose internalized in HepG2 cell monolayers after different time of exposure to the three insoluble ENMs. The results revealed significantly different adsorption profiles, allowing to accordingly tune the stickiness parameter of the models for each ENM and thus to improve the capability of the GUI in determining the cumulative effective dose reaching cells as a function of the initially administered dose.

Further *in vitro* testing is ongoing for relating effective dose predictions with corresponding biological effects, refining the dose-response characterization, and establishing the proposed tool as the basis for a reliable alternative to expensive and ethically sensitive animal experiments.

References

DeLoid, G., Cohen, J., Pyrgiotakis, G. et al. (2015). Advanced computational modeling for in vitro nanomaterial dosimetry. *Part Fibre Toxicol 12*, 32. doi:10.1186/s12989-015-0109-1

Poli, D., Mattei, G., Ucciferri, N. et al. (2020). An integrated in vitro-in silico approach for silver nanoparticle dosimetry in cell cultures. *Ann Biomed Eng 48*, 1271-1280. doi:10.1007/s10439-020-02449-5

Sohal, I., O'Fallon, K., Gaines, P. et al. (2018). Ingested engineered nanomaterials: State of science in nanotoxicity testing and future research needs. *Part Fibre Toxicol 15*, 29. doi:10.1186/s12989-018-0265-1

Thomas, D., Smith, J., Thrall, B. et al. (2018). ISD3: A particokinetic model for predicting the combined effects of particle sedimentation, diffusion and dissolution on cellular dosimetry for in vitro systems. *Part Fibre Toxicol 15*, 6. doi:10.1186/s12989-018-0243-7

Presentation: Posters

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TargetTri: Safety assessment and de-risking of novel drug targets

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Assessing safety liabilities of (exploratory) drug targets in the early preclinical phase of development is an important aspect of managing the drug discovery pipeline. On the one hand, toxicity information is imperative for the de-risking of therapeutic targets that are progressing through the development pipeline. On the other hand, safety considerations can aid in the prioritization