



University of Genova

**Department of Earth, Environmental
and Life Sciences**

**Doctorate Course in Earth and
Environmental Science and
Technology**

Università degli Studi di Genova



Dottorato in Scienze e Tecnologie
per l'Ambiente e il Territorio

Curriculum in biology applied to agriculture and the environment

Research Theme n.

Titolo: Implementazione di modelli in vitro 2D e 3D umani per lo studio dei meccanismi di tossicità da inalazione di fibre minerali e l'individuazione di biomarcatori specifici del potenziale cancerogeno Title: Implementation of 2D and 3D human in vitro models for the study of the toxicity mechanisms of mineral fibre inhalation and the identification of specific biomarkers of carcinogenic potential
Tutor: -Prof. Sonia Scarfi (UNIGE)
Program description including the formation program abroad The project focuses on the implementation and validation of 2D and 3D in vitro models of human lung tissues to understand the biochemical mechanisms of toxicity of different asbestos fibers and also for the prediction of the toxicity/carcinogenicity derived from their prolonged inhalation. This type of particulate causes well known health pulmonary problems such as lung fibrosis, asbestosis, lung cancer and pleural mesothelioma. In nature, the occurrence of numerous minerals with asbestiform behavior has been widely documented. These are fibrous, friable silicates, able to split longitudinally into needle-like micro fragments that can be inhaled, also containing heavy metal impurities (e.g. Fe, Mn, Cr), resulting in different biodegradability in vivo, which ultimately cause environmental and workplace biosafety problems of great relevance. The project will be developed in phases by testing in vitro models of increasing complexity, from simple co-culture systems of human lung cell types relevant to the development of inflammatory/carcinogenic pathways (i.e., alveolar macrophages, epithelial, endothelial, mesothelial cells) to the use of 3D lung reconstructed tissues models recapitulating faithfully the human bronchial/alveolar tissue architecture. Regarding the 2D in vitro models they'll be focused on the biochemical mechanisms of toxicity of different asbestos fibers (aragonite, crocidolite, and chrysotile) in the macrophage-mesothelial cell axis, attempting to trace these toxicity mechanisms back to the different chemical and physical characteristics of the fibers. The cellular models used will be human THP-1 macrophages and human mesothelial cells of the MET-5a lineage. The endpoints that will allow to understand the different toxicity mechanisms and build biochemical models of the alterations occurring in the cells, will be: acute and chronic cytotoxicity in macrophages; acute and chronic cytotoxicity in mesothelial cells; Chronic alteration of antioxidant defenses; Mitochondrial damage and mitochondrial oxidative stress; Lysosomal damage; DNA damage on cells surviving long term exposure; determination of intracellular concentration of

heavy metals released from fibers; Chronic inflammation through gene expression studies. Regarding the implementation and validation of the 3D lung tissue in vitro models, although perfectly mimicking the micro-physiology of the lower respiratory tract and of the lung parenchyma, they lack the immune cell component essential for the development of acute and chronic inflammatory responses at the basis of the fibrotic and carcinogenic processes in the lung. As such, in the last phase of the project the immune cells will be added to 3D reconstructed tissues to complete the models for the toxicity/carcinogenic predictive studies. The functionality of the models will be tested by using positive and negative mineral fibre controls of carcinogenicity in humans. The following parameters will be investigated to confirm/disprove the validity of the predictive model: short- and long-term cytotoxicity, oxidative stress, genotoxic stress, production of acute and chronic inflammatory cytokines, expression of epithelial-to-mesenchymal transition markers and transformation potential.

If the models prove to be reliable, they could be used as predictive tools for the biosafety assessment of chemical substances, mineral fibres and environmental particulates potentially inhalable by humans, also helping to understand their mechanisms of toxicity.

Period abroad:

For a period of no less than three months, the PhD candidate will carry out part of the experimental thesis in the research laboratories of the Mattek in vitro Life Science Corporation (Bratislava, Slovakia), a leading company in the development of 3D human microtissue models, under the tutorage of Dr Jan Markus, for the implementation of the EpiAirway™ and EpiAlveolar™ models by the addition and further testing of the immune cell component to the respective in vitro systems.

Financial support:

PRIN2022 "SEEDS" overheads, 100022-2023-SS-PRIN_Bando2022, sufficient to cover the costs of a personal pc and of the reagents for the development of the research project.

Tutor's publications in the last 3 years:

-Castellano M., Doderio A., **Scarfi S.**, Mirata S., Pozzolini M., Tassara E., Sionkowska A., Adamiak K., Alloisio M., Vicini S. Chitosan–Collagen Electrospun Nanofibers Loaded with Curcumin as Wound-Healing Patches. *Polymers*, 2023, 15(13): 2931. DOI: 10.3390/polym15132931

-Mirata S., Asnaghi V., Chiantore M., Salis A., Benvenuti M., Damonte G., **Scarfi S.** Photoprotective and Anti-Aging Properties of the Apical Frond Extracts from the Mediterranean Seaweed *Ericaria amentacea*. *Marine Drugs*, 2023, 21(5): 306. DOI: 10.3390/md21050306

-Gualtieri A.F., Mirata S., Almonti V., Bassi A.M., Meo C., **Scarfi S.**, Zapparoli M., Armeni T., Cianfruglia L., Marzioni D., Fantone S., Tossetta G., Stipa P., Laudadio E., Sabbatini S., Minelli C., Di Valerio S., Vaiasicca S., Procopio A.D., Pugnali A. In vitro toxicity of short vs long chrysotile fibres. *Periodico di Mineralogia*, 2023, 92(2): 203-222. DOI: 10.13133/2239-1002/18012

-Bassi A.M., Mirata S., Almonti V., Tirendi S., Vernazza S., Fornasini L., Raneri S., Bersani D., Passalacqua M., Gualtieri A.F., **Scarfi S.** Cytotoxic and pro-inflammatory early effects of mineral fibres on human alveolar epithelial and immune cells. *Periodico di Mineralogia*, 2023, 92(2): 223-239. DOI: 10.13133/2239-1002/18082.

-Gualtieri A.F., Leoncini M., Fantone S., Valerio S.D., Tossetta G., Procopio A.D., Marzioni D., Pugnali A., Bassi A.M., Almonti V., Mirata S., Vernazza S., Tirendi S., Marengo B., Traverso N., Passalacqua M., **Scarfi S.**, Raneri S., Fornasini L., Bersani D., Perchiazzi N., Ballirano P., Pacella A., Bloise A., Ottaviani M.F., Mattioli M., Giordani M., Ventura G.D. PRIN 2017 Fibres - A Multidisciplinary Mineralogical, Crystal-Chemical and Biological Project. What have we learned after four years of research? *Periodico di Mineralogia*, 2023, 92(2): 143-158. DOI: 10.13133/2239-1002/18021.

-Benvenuti M., Di Piazza S., Salis A., Cecchi G., Zotti M., **Scarfi S.**, Damonte G. A novel method for the extraction and characterization of metabolites from Basidiomycota: *Pleurotus ostreatus* (Jacq.) P. Kumm., 1871 as a case study. *Sep Sci Plus*, 2023, 10(6): e2300116. <https://doi.org/10.1002/sscp.202300116>.

-Raneri S., Gianoncelli A., Bonanni V., Mirata S., **Scarfi S.**, Fornasini L., Bersani D., Baroni D., Picco C., Gualtieri A.F. The influence of cation exchange on the possible mechanism of erionite toxicity: A synchrotron-based micro-X-ray fluorescence study on THP-1-derived macrophages exposed to erionite-Na. *Environ Res.* 2024, 252: 118878. doi: 10.1016/j.envres.2024.118878.

- Almonti V, Vernazza S, Mirata S, Tirendi S, Passalacqua M, Gualtieri AF, Di Giuseppe D, **Scarfi S**, Bassi AM. Toxicity and inflammatory potential of mineral fibres: The contribute of released soluble metals versus cell contact direct effects. *J Appl Toxicol*. 2024, 44(8): 1166-1183. doi: 10.1002/jat.4610.
- Bassi S, Benvenuti M, Mirata S, Di Piazza S, Salis S, Damonte G, Zotti M, **Scarfi S**. Enhanced antioxidant and anti-inflammatory activity of the extracts of *Pleurotus ostreatus* edible mushroom grown on *Lavandula angustifolia* residues. *Food Bioscience*, 2024, 60: 104382. doi: 10.1016/j.fbio.2024.104382.
- Gualtieri AF, Ferrari E, Rigamonti L, Ruozi B, Mirata S, Almonti V, Passalacqua M, Vernazza S, Di Valerio S, Tossetta G, Vaiasica S, Procopio AD, Fazioli F, Marzoni D, Pugnali A, **Scarfi S**. Bridging the gap between toxicity and carcinogenicity of mineral fibres by connecting the fibre parameters to the key characteristics of carcinogens: A comprehensive model inspiring asbestos-induced cancer prevention strategies. *Curr Res Toxicol*, 2024, 7: 100202. Doi: 10.1016/j.crttox.2024.100202.
- Scarfi S**, Almonti V, Mirata S, Passalacqua M, Vernazza S, Prakash Patel J, Brook M, Hamilton A, Kah M, Gualtieri AF. In vitro cyto- and geno-toxicity of asbestiform erionite from New Zealand. *Env Res*, 2025, 265: 120415. Doi: 10.1016/j.envres.2024.120415.
- Mirata S, Almonti V, Passalacqua M, Vernazza S, Bassi AM, Di Giuseppe D, Gualtieri AF, **Scarfi S**. Toxicity of size separated chrysotile fibres: the relevance of the macrophage-endothelial axis crosstalk. *Toxicology*. 2025, 511: 154032. doi: 10.1016/j.tox.2024.154032.
- Ballirano P, Pacella A, Mirata S, Passalacqua M, Di Carlo MC, Arrizza L, Montereali MR, **Scarfi S**. Fibrous erionite modifications following THP-1 macrophage phagocytosis: An insight into the mechanisms of interaction with biological systems. *J Hazard Mater*. 2025, 489: 137546. doi: 10.1016/j.jhazmat.2025.137546.
- Mihaly Cozmata L, Peter A, Nicula C, Uivarasan A, Szakács Z, **Scarfi S**, Moussa M, Mirata S, Bernárdez Fajardo P, Baldassari S, Drava G, Mihaly Cozmata A. Influence of *Alaria esculenta* powder on sensory acceptance, nutritional aspects, and staling of wheat bread. *Future Foods*, 2025; 12: 100805. <https://doi.org/10.1016/j.fufo.2025.100805>
- Giordani M, Mirata S, **Scarfi S**, Passalacqua M, Fornasini L, Drava G, Meli MA, Roselli C, Mattioli M. The cytotoxic/genotoxic role of impurities in soluble minerals: The case of natural (fibrous epsomite) versus synthetic (Epsom salt) magnesium sulphate. *Sci Total Environ*. 2025, 966: 178666. doi: 10.1016/j.scitotenv.2025.178666.
- Gualtieri AF, Mirata S, Passalacqua M, Bassi AM, **Scarfi S**. In vitro testing of hazardous mineral fibres: The issue of the concentration metric. *Environ Toxicol Pharmacol*. 2025, 119:104813. doi: 10.1016/j.etap.2025.104813.
- Mattiello A, Barbaglio G, Magnone M, **Scarfi S**, Vernazza S, Mirata S, Markus J, Letasiova S, Riva D, Premoli R. Preliminary evaluation of an extract of *Amaranthus caudatus* as a novel nutraceutical: effects on cholesterol and glucose absorption. *Front Nutr*. 2026; 12:1740097. doi: 10.3389/fnut.2025.1740097. PMID: 41601868; PMCID: PMC12833965.
- Moussa M, Mirata S, Moni L, Asnaghi V, Alloisio M, Pettineo S, Castellano M, Vicini S, Chiantore M, **Scarfi S**. Structural Characterization and Anti-Inflammatory Properties of an Alginate Extracted from the Brown Seaweed *Ericaria amentacea*. *Mar Drugs*. 2026; 24(1):41. doi: 10.3390/md24010041. PMID: 41590738; PMCID: PMC12842944.