Learning Scenario 5. Toxicology and *in vitro* models (course)

A course on *in vitro* toxicology started with the academic year 2015/2016, first semester, in the University of Milan, in the second year of the master degree of Veterinary Biotechnology Science. The course responds to the need to train students within Three Rs during the university career. "Toxicology and *in vitro* models", is a mainly practical course, while the theoretical part is only for support, in order to provide students with the knowledge of fundamental *in vitro* toxicity tests, information on new methodologies and applicability domain.

The course is three credits, 30 hours, inclusive of six hours of lectures and 24 hours of practice. The purpose of the course is to provide tools and information, on *in vitro* tests and models for toxicological studies, with particular attention on emerging techniques. The theoretical part was focused on the general description and the possible application of the methodologies in different areas of toxicological science, and future perspectives.

Moreover, an approach of comparison between *in vitro* and *in silico* data have been provided, through the intervention of experts. The most important part was represented by the practical in the laboratory, specifically drawn for student activity. Protocols on cytotoxicity assays, toxicity studies with *in vitro* epithelial barrier and permeability studies, *in vitro* models for endocrine disruptor activity, *in vitro* models for xenobiotics metabolism were provided to the students during the course.

The students are divided into groups of 4-5, and the protocols are executed with tutor help. Students first become familiar with classical cytotoxicity assays *i.e.* cytokines release (IL-1alfa, TNF-alfa), LDH release, MTT test, NRU test and subsequently they learn the use of different *in vitro* models from 3D to epithelial barriers and their applicability in toxicology. *In vitro* study on metabolism and permeability studies are outlined. For each topic a specific protocol is provided and all the practical activity is set-up by mimicking the real test, so that students can actually work and learn from the practical point of view.

The lab activity is environmentally friendly, as, where possible, material used during the activity is re-used. Through a meeting of experts, an approach of comparison between *in vitro* and *in silico* data was also addressed. The didactic material, inclusive of photographic material of the lab-activity related to the topic, is up-loaded on a dedicated on-line platform (www.ariel.unimi.it), available for the students, updated by the course teacher. The results of the examinations at the end of the course have shown a remarkable learning in particular in the choice and the manner of application of these methodologies.

Subject	**Toxicology and in vitro models**
Author/owner/ possible copyright issues	https://www.unimi.it/ Prof Francesca Caloni: francesca.caloni@unimi.it
Topics	*In vitro* models in toxicology, *in vitro* toxicity tests and applicability domain, practical approach in lab on the fundamental *in vitro* toxicity assays.
Eligible student level	Bachelor degree.
Teaching time	30 hours- three hours per week during the first semester from October to December.
Examples of online teaching material	Social media to connect with experts, Videos (Jove), link to suggested websites.
Examples of offline teaching material	Papers, textbook, discussion groups, meeting with experts, power point presentations.

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	Students visit also an Italian Lab working on *In vitro* Toxicology.
Helpful resources	https://www.unimi.it/ https://www.unimi.it/it/ugov/person/francesca-caloni Slides and presentations on institutional repository: https://fcalonitmv.ariel.ctu.unimi.it/v5/home/Default.aspx
Licenses, certification or accreditation	Università degli Studi di Milano, Veterinary Biotechnology Sciences (Class LM9), second year, first semester, 3 credits.
Integration in curriculum	This module is integrated in the curriculum Master Degree in Veterinary Biotechnology Sciences, Università degli Studi di Milano. It is a mandatory course, second year, first semester. The course can be followed also as a stand-alone course.
Examination	Oral examination.
Aims and learning objectives / outcomes	Concept of Three Rs, Replacement and role of in vitro approach. Knowledge of the principal *in vitro* tests/ assays. Information on the use of the *in vitro* methods in toxicology. Learn the applicability of different *in vitro* models in toxicology and the specific end points. Practical activity in lab with specific *in vitro* protocols. Learn about innovative methodologies, strategies and future perspective (i.e. spheroids, organoids).
Activities/ programme	Lectures Introduction to *in vitro* Toxicology (1 hour). In vitro models and evaluation of bioavailability, transport, metabolism and toxicity (4 hours). Nanotoxicology: *in vitro* toxicity of nanoparticles (1 hour). Practicals Cytotoxicity assays: release of LDH, MTT test, Neutral Red Up-take (6 hours). Evaluation of the release of interleukin 1 alpha (2 hours). Evaluation of the release of TNF-alpha (2 hours). Toxicity studies in vitro with 3D models (4 hours). Epithelial barriers and in vitro permeability studies (4 hours). Evaluation of TEER (Trans-Epithelial Electric Resistance) (2 hours). Endocrine Disruptor and in vitro models (2 hours). In vitro hepatotoxicity and metabolism (2 hours).
Assignment	Self-study: prepare/read provided documents/teaching materials. Attending students prepared a video on an emerging topic of an *in vitro* toxicology (Group work).
Student and teacher feedback	Mandatory Institutional Questionnaire filled in by the students. Compulsory Institutional Questionnaire filled in by the teachers. Optional Questionnaire created by the teacher for the students to improve the quality of the course. Published abstract presented in EUSAAT or World Congress on Alternative Methods in collaboration with the students.

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Helpful Resources	Caloni F. Three Rs education in veterinary science: a course on *in vitro* toxicology - 20th European Congress on Alternatives to Animal Testing, Linz, Austria, 24-27 August 2016; 17th Annual Congress of EUSAAT. In: ALTEX proceeding, 5 (1), p.30, 2016. F. Caloni, F. Danelli, S. Festa, F. Genova, V. Granata, F. Grassi Scalvini, S. Morelli, C. Pelizzoni, L. Schiavone, V. Bassi Pilot course on *in vitro* models in toxicology and students feedback tests, 2017 Altex Proceedings. - p. 181 Caloni F., Bertero A. The Role of the University in Teaching and Education on ALTernative : ROUTE ALT, 2018 ALTEX Proceeding Linz EUSAAT 2018 p 31.