UPPSALA UNIVERSITET

Degree projects and internships in *in vitro* environmental toxicology for fall 2023

The Drug Safety and Toxicology group at the Department of Pharmaceutical Biosciences offers degree projects and internships to interested students. Please, consult the next page for a summary of our activities.
Individual projects may comprise all degree projects (Examensarbete; 15/30/45/60 hp) and internships (Forskningspraktik, Projektarbete). We also offer a combined internship + thesis program for summer 2023, allowing interested candidates to learn applied methods before starting the thesis project. Degree projects are provided within the following subjects:

***De novo* establishment of *in vitro* fish bioassays via Crispr/Cas-mediated reporter gene knock-ins for pro- and retrospective environmental toxicity testing.**

Human toxicology has undergone a paradigm shift in testing, commonly known as "toxicology in the 21st century", replacing rodent-based *in vivo* tests with human cell line-based *in vitro* methods, given that the differences between species introduced substantial bias. However, in environmental toxicology, where the protection goal is primarily aquatic species, toxicity testing is still dominated by *in vivo* methods, leading to several ethical, economic, and technical issues regarding high-throughput screenings.
In parallel to human toxicology, environmental toxicology has seen the advent of several *in vitro* methods, such as reporter gene assays, which can record the molecular activation of toxicity pathways of primary health concern. However, due to technological drawbacks, environmental toxicologists rely on either human cell assays or chimeric systems that employ human genetic regulatory elements. The latter raises the question of the ecologic relevance of mentioned assays.
This project will employ the Crispr/Cas endonuclease technology to create endogenously regulated reporter gene assays in zebrafish cell lines. The Crispr/Cas technology enables a locus-specific genome alteration with high locus resolution. Instead of transfecting an entire reporter gene cassette with artificial regulators, only the reporter gene sequence itself will be introduced adjacent to loci that natively regulate toxicity pathways of primary concern.
The student will potentially be involved in permanent cell culture cultivation, Crispr/Cas-mediated cell line transgenesis, and testing promising clones with established reference compounds in a high-throughput setup.

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Development of multiple-endpoint bioassays for the assessment of environmental pollutants

Bioassays derived from permanent cell lines can screen an integrated effect of an environmental sample and, thus, disclose known, unknown, and mixture toxicity. However, toxicity can manifest in diverse ways, also on the cellular level. Currently, non-specific, specific, and reactive toxicity are monitored via different assays by recording various toxicity endpoints. Hence, one specific test is needed for every endpoint. Instead, we aim to "multiplex" endpoints within a single bioassay. Thus, one experiment and one microtiter plate would be sufficient for recording several toxicity endpoints. This study is a pilot project to develop and test multiplex-bioassay in permanent cell lines focused on physiologically essential endpoints such as cellular membrane stability, metabolism, and proliferation.
The student will be potentially involved in permanent cell culture cultivation, multiplexing of several endpoint assays, and assessment with known cytostatic drugs.

Keywords: toxicity pathways, cell culture, Crispr/Cas, acute cytotoxicity, environmental toxicology, bioanalytical toxicology

Methods: cell culturing and maintenance, reporter-gene assays, transgenesis, enzymatic assays, viability assays

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About the Department of Pharmaceutical Biosciences, Uppsala University

Our mission is to lead in research that benefits the development of effective and safe pharmaceuticals. We aim to increase the knowledge about the uptake, pharmacokinetics, metabolism, effects, and side effects of pharmaceutical drugs. For more information, please consult our homepage at https://www.farmbio.uu.se/

About the Drug Safety & Toxicology Group

The research group focuses on mechanisms of adverse health effects induced by pharmaceuticals, various environmental contaminants, and traditionally used medicinal plants. We are involved in projects mainly covering the following areas of toxicology: genetic toxicology, toxicity and metabolic

bioactivation, neurotoxicity during development, neurotoxicity and parkinsonism, and neuroblastoma and angiogenesis. Our main objective is to contribute with knowledge leading to improved hazard identifications and toxicological risk assessments when it comes to human exposures to various types of xenobiotics. For further information, please consult our homepage at https://www.farmbio.uu.se/research/drug-safety-/