ALTERNATIVES
RESEARCH & DEVELOPMENT
FOUNDATION

2021 ARDF GRANT RECIPIENTS

Established in 1993, the ARDF has been a mainstay of support for developing alternatives to animal-based methods in science. Through grant programs, achievement awards, and sponsorship of scientific conferences, ARDF advances high quality scientific research that aims to replace and reduce the use of animals.

1 CHARU CHANDRASEKERA, PHD; JASON O'BRIEN, PHD; & CLEMENS WITTWEHR, MS

University of Windsor; Windsor, Ontario, Canada

Machine-Readable AOP Evidence Model: Accelerating Pathway-Based Toxicity Testing

The landmark report, Toxicity Testing in the 21st Century, envisioned a future away from descriptive whole animal studies to one that embraces predictive risk assessment based on a mechanistic understanding of toxicity pathways. Central to this pathway-based paradigm is the adverse outcome pathway (AOP) framework—a systematic analytical framework that portrays sequential biological events causally linking the chemical properties of a substance to an adverse health outcome or ecotoxicological effect. While the AOP framework has gained tremendous traction over the last decade, its adoption by the scientific community and for regulatory risk assessment has been extremely limited. This is due, at least in part, to the lack of an appropriately structured machine-readable database with seamless interoperability among prominent global toxicological databases. The current AOP Knowledgebase (AOP-KB) that houses an inordinate amount of information critical for the AOP framework is primarily text-based. Therefore, it is essential to make the large amount of knowledge captured in the current AOP-KB highly accessible—evolve infrastructure to support modern chemical safety evaluation. Our goal is to create a "Machine Readable AOP Evidence Model" that will be built as a modular upgrade to the existing AOP-KB data model—to design an enhanced, more structured data model that will specifically advance information accessibility, findability, interoperability, and useability. We predict these efforts are essential to enable better integration of animal-free methods to support data-driven toxicology applications and regulatory decision making. We believe the work proposed herein to upgrade the AOP-KB—the single most critical platform in pathway-based toxicity testing—will expedite the development of Integrated Approaches to Testing and Assessment, in turn accelerating the pace of animal replacement.

2 SARA FORTUNA, PHD & BARBARA MEDAGLI, PHD

Univ. of Trieste (Trieste, Italy) and Italian Institute of Technology (Genoa, Italy)

De-novo engineered antibody fragments: validation of the first in-silico pipeline for antibody discovery

The increase of applications involving immunoreagents as tools in diagnosis, therapeutics, and research is boosting the need of new antibodies typically derived from in vivo and in vitro techniques. The development of computational methods for antibody generation and maturation aims to eliminate the need of animals along the whole process. Our team, committed to the task, has recently developed an enhanced computational protocol to generate new antibody fragments (VHH) against, potentially, any target without relying on animal immunization, animal-derived libraries, and reagents. Indeed, the protocol has been applied for the de-novo generation of VHHs against two targets of interest. Preliminary results showed that functional VHH could be successfully cloned and expressed. Now it is time to fully validate the in silico procedure with: (i) the throughout characterization of the recombinant VHHs; (ii) the VHH/target affinity evaluation with state-of-the-art protein-protein interaction methods, and (iii) the determination of the VHH/target three dimensional structure by X-ray crystallography to study their interactions at the atomic level and confirm the in silico predictions. The throughout characterization of the first computationally generated VHHs will send a strong message to the scientific community by setting an important milestone and leading the way in the animal-free generation of antibodies.

3 ALEXANDRA MAERTENS, PHD

Johns Hopkins University, Baltimore, MD

Mapping In Silico Sensitization Model Blind Spots with Cheminformatics

Of the 80,000 chemicals in commerce in the U.S., very few have safety data available - approximately 85% are untested for chemical safety, leaving humans exposed to thousands of chemicals with unknown hazard. While one approach to solving this is to increase the use of animal testing, this is both lengthy and costly, and the results do not always translate to humans, leading to uncertainty about the true hazard of a chemical. As a consequence, alternative methods are urgently needed to increase the speed, reduce the cost, and improve the relevance and accuracy of chemical toxicity assessments. The quickest and easiest method to predict chemical toxicity is with in silico models, which in the case of skin sensitization can achieve accuracy comparable to animal models, although both animal models and in silico models have known blind spots. We hope to improve the accuracy of existing models of skin sensitization so that they can be used more confidently, and extend this approach to respiratory sensitization, which has been an emerging concern and an endpoint that currently lacks a validated animal model. Therefore, we believe this is an opportunity to showcase the usefulness of building in silico models based on human data - not only because in silico models are quicker and cheaper than in vivo testing, but can be used proactively to help design safer alternatives.

4 ALYSHA SIMMONS, PHD & PHILLIP CLAPP, PHD

The University of North Carolina at Chapel Hill, Chapel Hill, NC

Development of an Organotypic Co-culture Model of Pediatric Exposure to Inhaled Pollutants

Acrolein is a ubiquitous air pollutant generated from a range of heating and combustion processes. Acrolein emission sources are well known; however, high dose and sub-chronic/chronic exposures pose an environmental justice issue because they disproportionately affect minority communities, low socioeconomic status areas, and children. Moreover, epidemiological studies continue to uncover health disparities in children, including asthma caused or exacerbated by second-hand smoke, pulmonary infection, and cardiovascular disease. Attempts to understand the mechanisms of toxicity associated with inhaled acrolein exposure have historically been performed in rodent models using intratracheal instillation of solubilized acrolein or inhalation chambers containing aerosolized acrolein. Unfortunately, rodent studies cannot provide translational data regarding age or genetic susceptibility or directly observe complex interactions in the bronchial respiratory tract. To address this gap in knowledge and decrease reliance on in vivo toxicity testing, we will use an open-source in vitro ALI co-culture model of the human bronchial epithelium developed to determine the suitability of primary human bronchial epithelial tissues for the incorporation of environmental justice and susceptibility modeling into inhalation toxicology. We will accomplish this by characterizing 1) functional endpoints of the epithelium, 2) the immune and pro-inflammatory response, and 3) markers of susceptibility to acrolein exposure. Characterization of this matched primary bronchial epithelial cell and fibroblast organotypic model would interrogate inter-donor variability in primary cell systems and yield the only cell-based model of inhaled chemical exposures in children. Further, all methods and data will be made publicly available to ensure the transparency and accessibility of a low-cost in vitro ALI co-culture model of the human bronchial respiratory tract to support its use to reduce animal testing.

5 MATHIEU VINKEN, PHD

Vrije Univeristeit Brussel, Brussels, Belgium

An animal-free approach for human safety testing of food additives

The use of animal tests for the assessment of human safety of chemicals is heavily criticized from the ethical, scientific, and economic point of view. The present project will provide a solution to this ubiquitous issue by introducing an animal-free, human-based, and cost-effective approach for the reliable prediction of adverse effects induced by chemicals. Focus will be put on food additives, of which some have been shown to cause liver toxicity in laboratory animals. This project will elucidate the mechanisms underlying these presumed liver toxic effects by incubation of these food additives in state-of-the-art spheroid cultures of primary human hepatocytes and non-parenchymal human liver cells. Through whole transcriptome templated oligo assay with sequencing read-out analysis and pathway analysis, a mechanistic scenario of the liver toxic effects induced by the food additives will be established. This will be substantiated by a series of translational and functional analyses of known triggers and key events in liver toxicity, in particular liver steatotic and cholestatic insults. Furthermore, the use of effects at the transcriptional level for setting limits for safe daily consumption of food additives will be explored. Overall, the outcome of this project will shed more light onto the liver toxic potential of food additives and will demonstrate the power of animal-free and human-based in vitro experimentation for chemical risk assessment purposes.

6 TRAVIS WALKER, PHD; TUGBA OZDEMIR, PHD; & TIMOTHY BRENZA, PHD
South Dakota School of Mines and Technology, Rapid City, SD

Development of a Physiologically-Relevant, Serum- and Animal-Free In Vitro Angiogenesis Assay

Angiogenesis is a complex and tightly controlled biological process of sprouting and growing new blood vessels from the existing vasculature. Essential in wound healing and in relieving ischemic tissues, angiogenesis is incremental in certain pathologies such as tumor growth, metastasis, and cardiovascular disease. Scientists continue to study angiogenesis in an effort to identify its impact on disease progression, to develop therapeutics, and to test drug-delivery vehicles. However, current in vitro models do not accurately recapitulate in vivo conditions, using undefined media constituents that may interact with exogenous drug-delivery vehicles and cytokines or that may alter cell responses. Further, in conjunction with other media additives that are undefined and vary between batches, all of the models use various angiogenic cytokines at different concentrations to induce angiogenesis. This lack of consistency forces researchers to rely on animal models to study drug-delivery mechanisms and tissue/cell interactions. Our research objective is to develop a robust, physiologically-relevant angiogenesis platform with controllable flow and cell organization. We can induce angiogenesis through a hydrogel medium, generating an engineered vascularized tissue. Our design allows us to easily incorporate other important in vitro models such as cancer-induced angiogenesis, drug delivery, and endothelium-induced inflammatory responses. We are preparing to investigate endothelial cell-macrophage interactions and the subsequent role in tissue healing and regeneration, as well as anti-cancer nanoparticle drug delivery in a fully perfusable, capillary-like system and a potential pro-inflammatory endothelial response upon particulate matter exposure. The resulting animal-free angiogenesis platform is specifically designed to accelerate the benchtop-to-bedside transition of biomedical technology and to promote the adoption of ethical, physiologically-relevant in vitro testing.

The Alternatives Research & Development Foundation expresses its appreciation to the Institute for In Vitro Sciences of Gaithersburg, Maryland, for its guidance in the scientific review process, and thanks all applicants who submitted proposals and others for their interest in developing alternative methods of conducting high quality scientific research.