TECHNICAL BRIEF

A Strategic Research Initiative on Creation of a Multimodal Platform for Genotoxicity Testing for Chemicals of Emerging Concern

A novel high-throughput platform utilizing genetically engineered cell lines with different metabolic capacity is urgently needed for fast and reliable genotoxicity screening for emerging chemicals of concern.

Introduction

Within the U.S. and globally, chemicals of emerging concern (CECs) from various sources, including new technologies like 3D printing, vehicle combustion, wildfires, textiles, furniture, pharmaceuticals, and cosmetics exist in both the indoor and outdoor environment.[1, 2] Released CECs such as endocrine disrupting chemicals, flame retardants (e.g., organophosphate flame retardants [OPFRs]), engineered nanomaterials, micro- and nano-plastics, and per- and polyfluoroalkyl substances (PFAS) can enter the human body directly through inhalation and dermal contact, or indirectly through contaminated food and water. There is growing concern over the health risks of these largely uncharacterized CECs due to increased release and ever-expanding inventory. Following the earlier collaborative efforts from the Contaminants of Emerging Concern Interagency Working Group (CEC IWG),[3, 4] the National Emerging Contaminants Research Initiative was established in 2022 by the White House Office of Science and Technology Policy and the CEC IWG to coordinate federal research on CECs to improve the identification, analysis, monitoring, and mitigation methods of CECs.[5]

Since the enactment of the Toxic Substances Control Act in 1976, U.S. EPA conducts compliance monitoring of only 4 out of the 86,000 existing chemicals registered for use in commerce, including polychlorinated biphenyls, asbestos, lead-based paint, and formaldehyde.[6, 7] Many of these untested existing chemicals are potential DNA damaging agents that promote cancer, neurological disorders, immune disorders, and aging, thereby placing many people, especially those from vulnerable populations, at risk during their daily work and personal lives. Therefore, it is critical not only to have rapid assays to identify DNA damaging chemicals, but also more in-depth information is needed on the mechanisms of action. Furthermore, evidence shows that differences in DNA repair capacity among apparently healthy populations impacts disease susceptibility. Therefore, it is important to identify DNA repair pathways in genetically engineered cells with varying DNA repair capacity that can ultimately be linked to personal susceptibility for precision prevention and treatment.

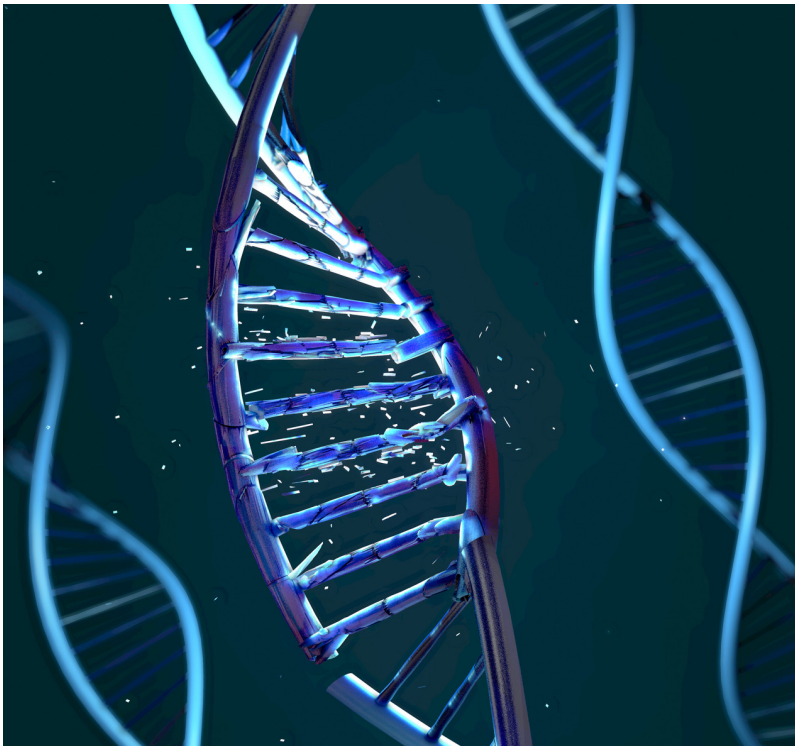
Chemical Insights Research Institute (CIRI) of UL Research Institutes' new research initiative aims to develop high-throughput methods for genotoxicity and toxicity testing of key CECs. Utilizing the CometChip™ System[8] we will develop a suite of genetically engineered cells wherein each cell line acts as an animate sensor for specific DNA damage. This will be made possible by querying which DNA repair pathways are activated. In addition to pathway identification, we will also exploit genetically engineered cell lines with different metabolic capacities so that we can learn how metabolism shapes risk. We will develop a new method to study the DNA damaging potential of CECs

that are research priorities for CIRI but may have unknown biological properties. The following research questions spanning several CIRI strategic research initiatives will be explored using the proposed platform:

1. PFAS Exposure and Human Health Research Questions:
    a. What forms of oxidative DNA damage are associated with PFAS exposure stemming from textile usage?
    b. Are there specific DNA repair pathways initiated by PFAS mediated DNA damage?
2. Furniture Flammability and Flame Retardant Exposure Research Questions:
    a. What are the human relevant levels of organophosphate flame retardants (OPFRs) that cause DNA damage and cellular injury?
    b. What types of DNA lesions are formed by OPFRs?
    c. What DNA repair pathways are elicited or impaired by OPFR exposure?
3. Wildland Urban Interface (WUI) and Wildfire Research Questions:
    a. How do hazardous air pollutants associated with wildfire smoke such as benzo[a]pyrene contribute to DNA lesion formation?
    b. How might wildfire particle pollution contribute to DNA repair activation?
4. Electronic Nicotine Delivery Systems (ENDS) and Vaping Research Questions:
    a. How do chemical byproducts of vaping, including nitrosamines, influence DNA damage?
    b. What frequency and vaping behavior induce DNA damage?

Study Objectives

- Utilize genetically engineered cells to perform DNA repair-trapping to assess the potential of CEC to create single strand breaks.
- Develop cell-based repair-trapping sensors to detect CEC mediated small base lesions and single strand breaks.
- Develop cell-based repair-trapping sensors to detect CEC mediated bulky lesions and reveal the potential relevance of metabolic activation.



Study Plan Overview

The study objectives will be achieved based on the following timeline of activities.

Year 1. Create genetically engineered CRISPR cell lines that can be used for repair-trapping of single strand breaks. Demonstrate efficacy of this approach with a proof-of-principal chemical (hexavalent chromium).

Year 2. Create genetically engineered cell lines that can be used for repair-trapping of small base adducts. Demonstrate efficacy for a CEC (specifically, NDMA). Publish protocols on the NextGen Protocols website.

Year 3. Create genetically engineered cell lines that can be used for repair-trapping of bulky adducts. Create multiple cell lines, each with different metabolic capacity. Perform proof-of-principal with the CEC benzo[a]pyrene. Publish protocols on the NextGen Protocols website.

Scientific Outcomes

The main scientific outcome of this platform will be the ability to rapidly identify genotoxic CECs at low levels that are commonly experienced by the general public. Current genotoxicity assessments require CEC concentrations that are higher than human relevant exposure levels to identify genotoxic agents. Thus, this collaborative effort will enhance our ability to detect hazardous CECs that alter DNA and potentially contribute to mutations leading to disease. A second outcome of this research will be determining how individuals with deficits in critical metabolic and DNA repair enzymes may be more susceptible to genotoxic CEC exposure, which may increase their risk and rate of disease development.

Research Partners

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