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Mapping ToxCast/Tox21 HTS Assay Endpoints to Key Characteristics of Carcinogens

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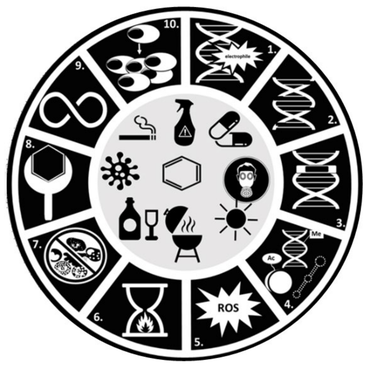
Background

- The **Key Characteristics of Carcinogens** (KCC, described in the figure below) framework was first conceptualized in 2016 by Smith et al. [1] through an analysis of Group 1 carcinogens identified by the International Agency for Research on Cancer (IARC) monograph program.
- This framework provides a **mechanistic approach** to evaluating potential cancer hazards.



KCC1: Is Electrophilic or Can Be Metabolically Activated to Electrophile
KCC2: Is Genotoxic
KCC3: Alters DNA Repair or Causes Genomic Instability
KCC4: Induces Epigenetic Alterations
KCC5: Induces Oxidative Stress
KCC6: Induces Chronic Inflammation
KCC7: Is Immunosuppressive
KCC8: Modulates Receptor-mediated Effects
KCC9: Causes Immortalization
KCC10: Alters Cell Proliferation, Cell Death, or Nutrient Supply

- **In vitro assay endpoints** can be mapped to the KCC framework through appropriate annotations, supporting the use of non-animal testing approaches to assess and predict potential carcinogenicity.
- **Efforts to develop and map relevant in vitro assays** that cover each mechanism in the KCC framework are or have been ongoing [2-4].

Goals

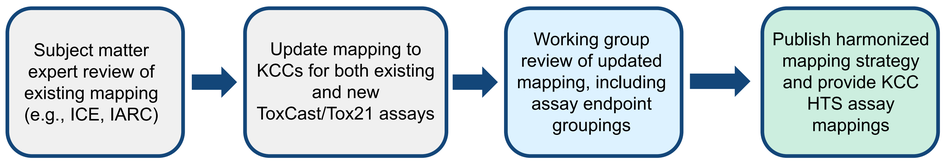
- **Update assay mapping** by incorporating the latest version of U.S. Environmental Protection Agency (EPA) data that include changes in assay nomenclature.
- **Expand previous efforts** to annotate ToxCast/Tox21 assays by mapping them to the KCC framework.
- **Engage experts** to review existing mapping, evaluate mechanistic relevance within the KCC framework, and provide recommendations.
- **Document mapping decisions** to ensure streamlined updates and promote consistent interpretation.

Previous Mapping Efforts

- The U.S. Environmental Protection Agency (EPA) Toxicity Forecaster (ToxCast) program consists of medium- and high-throughput screening (HTS) assay data aggregated from 20+ sources, including the Toxicology in the 21st Century (Tox21) federal collaboration, which includes data from nearly 10,000 chemicals [5].
- For this project, data were obtained from ToxCast's latest invitrodb version 4.1 [6].
- Existing KCC assay mappings from IARC and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) Integrated Chemical Environment (ICE) database [7,8] served as a starting point for this work.

Expert Working Group Composition and Workflow

- Our **workflow** included subject matter expert review of existing and updated KCC mapping for ToxCast/Tox21 assays. This, guided expert working group discussions to develop a harmonized mapping approach and assess assay relevance to carcinogenicity.



- Our **expert working group** included 20 scientists from diverse institutions:
  - California Office of Environmental Health Hazard Assessment
  - EPA
  - IARC Monographs Program
  - National Institute of Environmental Health Sciences (NIEHS)
  - NICEATM
  - Texas A&M University
  - University of California, Berkeley

Current Mapping Approach

Tiering approach:

- Mapping assays to a single KCC is challenging, as many assays often measure general bioactivity and can inform one or multiple KCCs. To address this complexity, a **tiering approach** was developed to differentiate whether assays reflect direct or indirect/downstream effects.
  - **Tier A: Direct Effect on KCC (higher mapping relevance)**
    Tier A mapping is based on the specific bioassay endpoint. For example, assays targeting **TP53** (tumor suppressor gene) map directly to **KCC2** (Is Genotoxic). Similarly, assays targeting **PGR** (progesterone receptor gene) map directly to **KCC8** (Modulates Receptor-mediated Effects), reflecting the close relationship between the endpoint itself and the KCC.
  - **Tier B: Indirect/Downstream Effect on KCC (lower mapping relevance)**
    Tier B mapping is based on bioassay endpoints that inform pathways or processes indirectly contributing to a KCC. For example, assays detecting decreases in **TP53 gene expression** map to **KCC3** (Alters DNA Repair or Causes Genomic Instability) since this informs on a pathway involved in DNA damage response. Similarly, assays measuring **Cyp1a1 gene expression** as a biomarker for AhR activation map to **KCC8**, reflecting indirect modulation of receptor-mediated effects.

Assay mapping recommendations:

- Mapping decisions were guided by overarching considerations and fundamental principles to ensure consistency and relevance in associating assays with KCCs.

	Recommendation

Directionality	• Within the new version of invitrodb (version 4.1) assays were treated as bidirectional; however, response direction must be carefully considered for specific KCC associations:
	  • **KCC3**: Only a decrease in DNA repair capacity or decrease in response (e.g., decrease in the concentration of a DNA repair enzyme) is relevant for cancer.
	  • **KCC4 (Induces Epigenetic Alterations)**: Both increases or decreases in response are linked on the effects of epigenetic changes and must be considered case-by-case among relevant assays.

Cytotoxicity assays and KCC10	• All viability assays characterizing cytotoxicity were previously mapped to KCC10 (Alters Cell Proliferation, Cell Death, or Nutrient Supply). • After discussion, the expert working group decided that these assays should not be mapped to KCC10, as this would overstate the assay response. Only assays where an increase in viability can inform on proliferative responses are included in the current mapping.
Mapping assays to KCC9	• Previous mapping included assays that were mapped to KCC9 (Causes Immortalization). • After review, the expert working group agreed that none of the assays in invitrodb v4.1 are relevant for KCC9.

Current Status and Next Steps

- The expert working group has reviewed approximately 800 assay endpoints to date.
- A complete review of all 1499 assay endpoints is expected by mid-2025.
- All group discussions will be documented and outcomes made publicly available to ensure full transparency.

Conclusion

Summary

- The updated mapping of the ToxCast/Tox21 assays to the KCC framework will enhance assay transparency and interpretability relative to the KCCs.
- A comprehensive list of KCC-mapped HTS assays will be made publicly available upon completion, supporting mechanistic cancer hazard assessment and advancing understanding of chemical-driven carcinogenesis.
- This work will be used to update the mechanistic assay interpretation available through ICE (https://ice.ntp.niehs.nih.gov/).

Integrated Chemical Environment

Perspective

- Data gaps still exist within the ToxCast/Tox21 program, and mechanisms relevant to KCC should be put into the context of overall carcinogenesis rather than considered in isolation.
- Tier A could encompass targeted and direct assays, while Tier B could encompass broader endpoints that require additional review by the user or stakeholder to determine appropriate application.
- This mapping is expected to support development of predictive computational approaches for chemical carcinogenic mechanisms.

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