4. Mechanistic and Other Relevant Data

4.3 Data relevant to comparisons across agents and endpoints

A vast amount of high throughput screening (HTS) data has been generated as part of the interagency Tox21 and USEPA ToxCast research programs. The USEPA has systematically analysed over 1 million concentration response sample-assay pairs from ToxCast and Tox21. The resulting concentration response models and activity calls have been released via the CSS ToxCast Dashboard (www.actor.epa.gov/dashboard). Summary matrix files, the toxcast data analysis pipeline (tcpl) R package and connected database (invitrodb_v1) have also been released (www.epa.gov/toxcast/data). Summary matrix files are consistently outputted with rows of chemicals, columns of assay endpoints and intersection of various models parameters (e.g., logAC50, top of curve), activity call, testing status or z-scores (i.e., potency distance from cytotoxicity burst). The tcpl R package and associated database enables access to all of the underlying concentration response data, the analysis decision logic and methods, concentration response model outputs, activity calls and activity caution flags.

Tox21 and ToxCast research programs have tested over 8000 and 1800 chemicals, respectively. ToxCast, specifically, has tested 1000 chemicals across the full assay battery in conjunction with ToxCast Phase I and II. The remaining 800 chemicals were tested as part of an endocrine profiling effort that resulted in a subset of assays being tested. Within the ToxCast Phase I and II chemical libraries, 41 organophosphate pesticides or their oxon metabolites were tested across the entire assay battery, including Diazinon, Malathion, Parathion, and Tetrachlorvinphos as well as three oxon metabolites, Diazoxon, Malaoxon and Paraoxon. Glyphosate was not included in either of the chemical libraries due to physico-chemical property constraints.

In order to explore the bioactivity profiles of these compounds and their potential impact on carcinogenic processes the 821 assay endpoints were grouped based on manual mapping to 10 Cancer Key Characteristics (CKC) (Appendix ZZ). Of the 821 assay endpoints, ~500 [finalize] assay endpoints were mapped to 7 of the 10 CKC [summary table].

1. Is Electrophilic or Can Be Metabolically Activated – 81 assay endpoints
2. Is Genotoxic – 14 assay endpoints
3. Alters DNA repair or causes genomic instability – 0 assay endpoints
4. Induces Epigenetic Alterations – 18 assay endpoints
5. Induces Oxidative Stress – 34 assay endpoints
6. Induces chronic inflammation – 48 assay endpoints

7. Is Immunosuppressive – 0 assay endpoints

8. Modulates receptor-mediated effects – 143 assay endpoints

9. Causes Immortalization – 0 assay endpoints

10. Alters cell proliferation/death or nutrient supply – 157 assay endpoints

The assay endpoints groupings were not intended to serve as definitive linkages to carcinogenic outcomes, but provide insight into the bioactivity profile of a chemical highlighting the chemical's potential to interact or disrupt targets biologically associated with cancer. The Toxicological Prioritization Index (ToxPi) visualization of the CKC assay endpoint groupings highlighted each chemical's relative potential to interact with the set of assay endpoints per grouping (Reif et al 2010). ToxPi were organized with each slice representing a CKC and generated for all chemicals in the 1000 ToxCast Phase I and II chemical library. The value underlying each slice was the activity concentration (i.e. AC50 represented as –log10(AC50/1000000) for active or 0 for inactive) averaged across all assay endpoints. The individual slice values were further normalized from 0 to 1 based on the range of responses for each slice across all 1000 chemicals.

The chemicals and resulting ToxPi outcomes have also been stratified with respect to IARC cancer classifications. Of the 1000 ToxCast Phase I and II chemicals and 843 IARC classified chemicals with an assignable CAS registry number, 180 chemicals overlapped and were therefore tested across the full ToxCast assay battery. Of the 180 chemicals, 8 were Group 1, 16 were Group 2A, 58 were Group 2B, 97 were Group 3, and 1 was Group 4. It should be noted that the ToxCast program has performed limited screens for genotoxicity as evidenced by the limited number of assay endpoints in CKC which primarily target p53 and are not direct direct measures of DNA damage (e.g., Ames assay). Therefore, there would be limited expectation that group 1, 2A and 2B classified compounds would necessarily have high CKC ToxPi values. Nonetheless, overall CKC ToxPi and by slice (i.e. CKC assay grouping) values provide insight into a chemical's potential to interact with cancer relevant targets as well as plausible mechanisms of action.