4.3   Data relevant to comparisons across agents and endpoints

4.3.1. General description of the database

High throughput screening (HTS) data generated by the Tox21 and ToxCast research programs of the US government were analysed to inform conclusions on in vitro bioactivity. The data considered included concentration response models and activity calls publicly released via the Chemical safety for Sustainability (CSS) ToxCast Dashboard of the US EPA (www.actor.epa.gov/dashboard). Summary matrix files, the toxcast data analysis pipeline (tcpl) R package and connected database (invitrodb_v1) were accessed from the US EPA public website on 3 March 2015 (www.epa.gov/toxcast/data). Summary matrix files gave, for each chemical, assay endpoints as well as 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 enabled access to the underlying concentration response data, the analysis decision logic and methods, concentration response model outputs, activity calls and activity caution flags.

The ToxCast program has tested approximately 1000 chemicals across the full assay battery comprising more than 800 *in vitro* tests. This included more than 30 organophosphate pesticides or their oxon metabolites, including diazinon, malathion, parathion, and as well as the oxon metabolites malaoxon and paraoxon. An additional 800 chemicals, including tetrachlorvinphos and the oxon metabolite diazoxon, were tested as part of an endocrine profiling effort that resulted in a subset of these assays. Glyphosate was not included in either of the chemical libraries due to physico-chemical property constraints.

MEETING DRAFT
Do not quote, cite, or distribute

Data on 821 assay endpoints derived from 558 assay components (i.e., readouts) and 342 assays (i.e., experiments) are available in the US EPA dashboards. The 342 assays were sourced from 7 vendors or collaborators spanning diverse technological and biological space, including over 300 gene targets. About half of the final assay endpoints were analysed from cell-free assay formats with the remainder from cell-based assays. It is of note that while the cell-based assays have a variable metabolic capacity, it is generally limited.

4.3.2. Aligning *in vitro* assays to 10 "key characteristics" of known human carcinogens

In order to explore the bioactivity profiles of the compounds under evaluation in Monograph volume 112 with respect to their potential impact on the mechanisms of carcinogenesis, the Working Group members performed mapping of the 821 available assay endpoints in Tox21/ToxCast to 10 Key Characteristics of known human carcinogens (REF to IARC instructions for Section 4 table). Independent assignments were made for each assay type to the one or more "key characteristics" based on the biological target being probed by each assay and the interpretation of the assay read-out. The consensus assignments comprise 274 assays that mapped to 7 "key characteristics" as shown below.

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

MEETING DRAFT
Do not quote, cite, or distribute

9.   Causes Immortalization – 0 assay endpoints

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

The match of an assay to the "key characteristic" were to provide insight into the bioactivity profile of a chemical highlighting the chemical's potential to interact or disrupt targets biologically associated with cancer. In addition, based on the in vitro assays that represent each "key characteristic", a comprehensive and unbiased evaluation of the relative potency of each compound under evaluation may be performed. For each assay, an activity concentration (*i.e.*, activity concentration exceeding +/-3 mean absolute error variability over the baseline in each assay) for active or 0 for inactive (assays where at no concentration tested the signal exceeded the threshold of +/-3 mean absolute error variability over the baseline) were derived. To integrate the data across individual assays into the cumulative signal for each "key characteristic", the ToxPi software (Reif et al. 2013) was used. ToxPi is an example of a prioritization support tool for integration of evidence across endpoints and to visualizing the relative prioritized ranks of the compounds under consideration. ToxPi was proposed by (Reif et al. 2010) as a dimensionless index score that enables integration of multiple sources of evidence on exposure and/or safety, transformed into transparent visual rankings to facilitate decision making. Different data are translated into ToxPi scores to derive slice-wise scores for all compounds as detailed below and in the publications describing the approach (Reif et al., 2010) and the associated software package (Reif et al., 2013). 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 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).
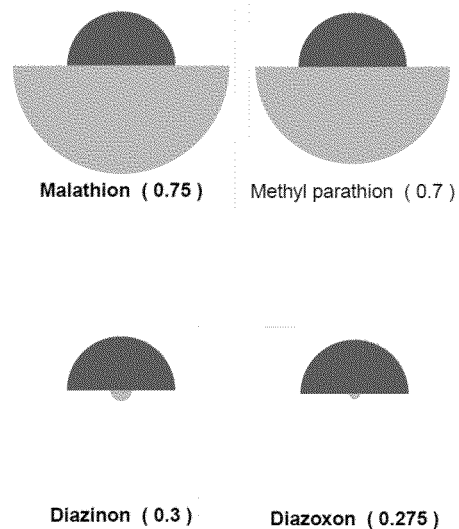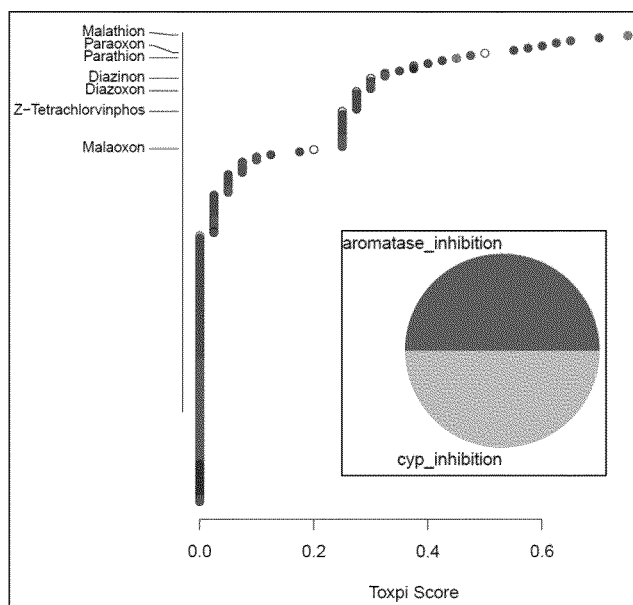
MEETING DRAFT
Do not quote, cite, or distribute
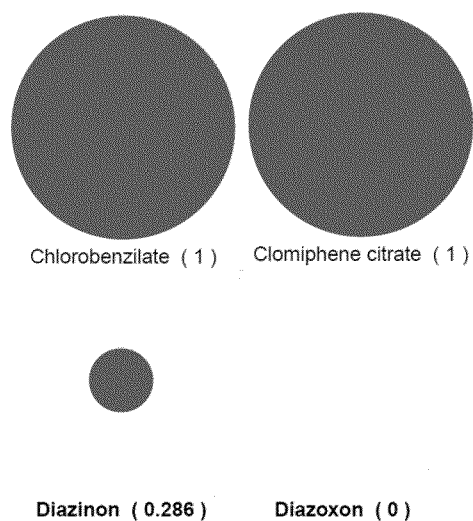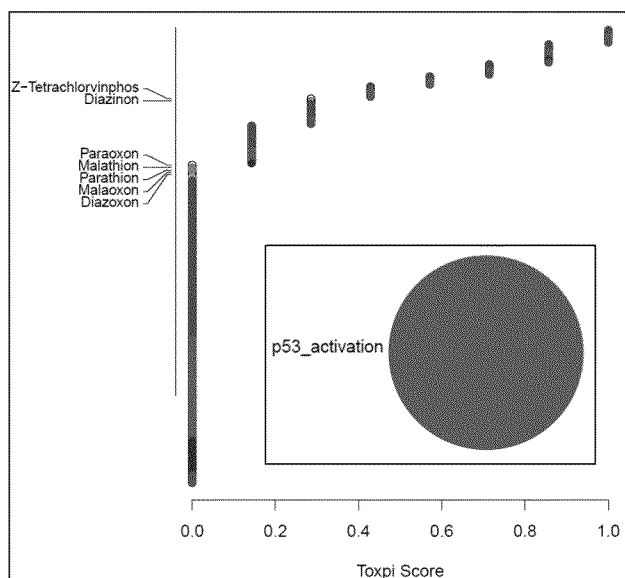
MEETING DRAFT
Do not quote, cite, or distribute

4.3.3. Diazinon-specific effects across 7 "key characteristics" based on *in vitro* screening data.

Relative effects of diazinon and diazoxon were evaluated compared to 180 IARC-evaluated chemicals that also were screened by Tox21/ToxCast program. 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.The results are presented below as a rank order (left hand side graph in Figures 1-8) of all compounds in the analysis (180 IARC-classified chemicals and monograph volume 112 compounds) arranged in the order of their relative effects (active, i.e., effects observed at any tested concentration, to inactive, i.e., no effect was observed at any concentration tested). The relative position of diazinon and diazoxon in the ranked list is also shown on the y-axis. The inset in the scatter plot shows the components of the ToxPi chart of the sub-categories that comprise assays in each characteristic, as well as their respective color-coding. On the right-hand side, two top-ranked chemicals in each analysis are shown to represent the maximum ToxPi score. The ToxPi charts of diazinon and diazoxon are shown below.

1. "**Electrophilic or ability to undergo metabolic activation.**" There were 31 assays mapped to this characteristic in sub-categories of CYP inhibition (29) and aromatase inhibition (2).
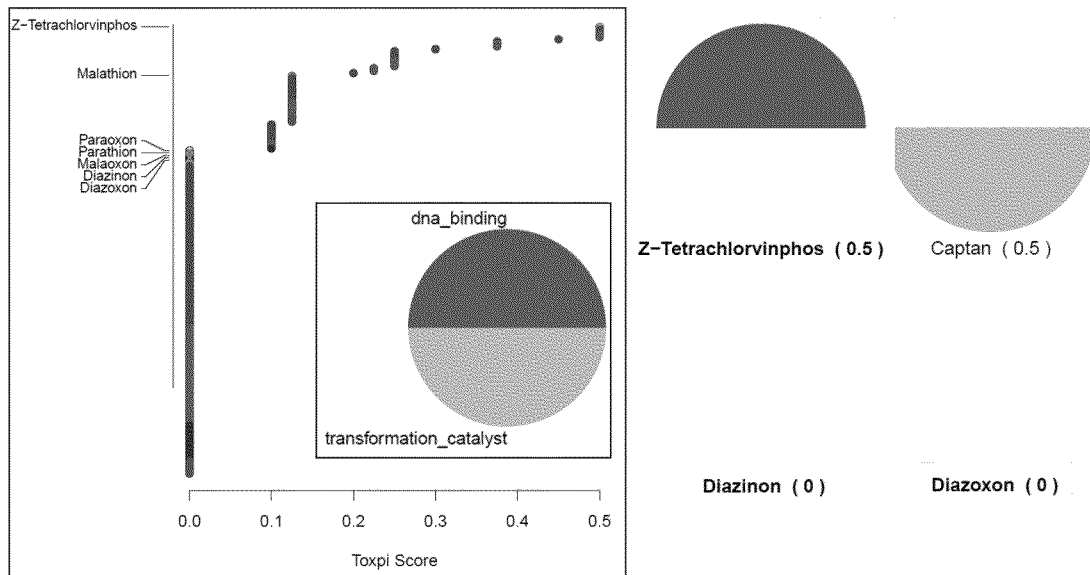
MEETING DRAFT
Do not quote, cite, or distribute

2. "**Genotoxic.**" There were 9 assays mapped to this characteristic all belonging to a sub-category of p53 activation.
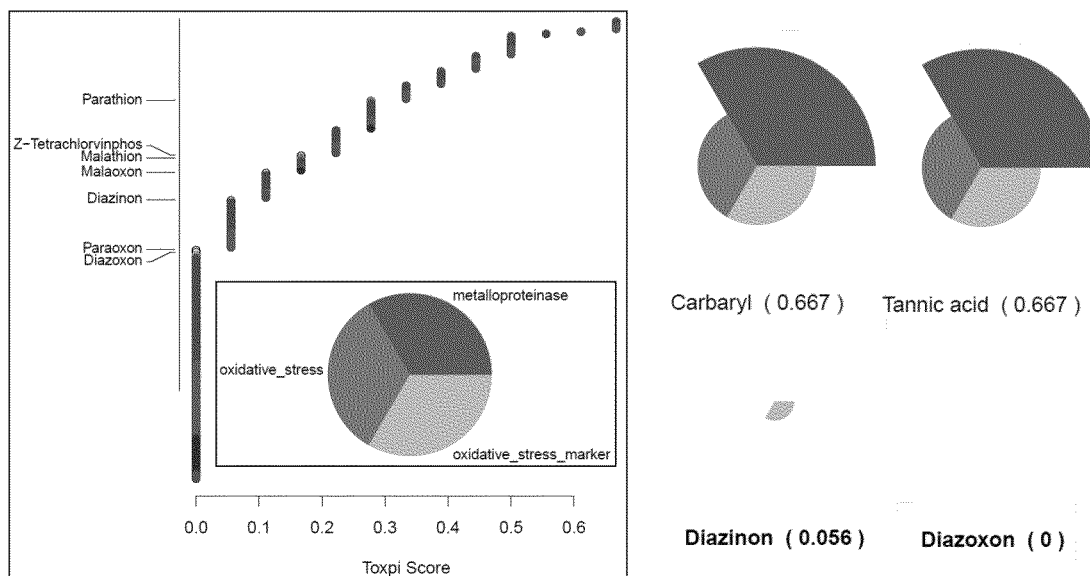

MEETING DRAFT
Do not quote, cite, or distribute

4. "**Epigenetic alterations**." There were 11 assays mapped to this characteristic in sub-categories of DNA binding (4) and transformation (7).
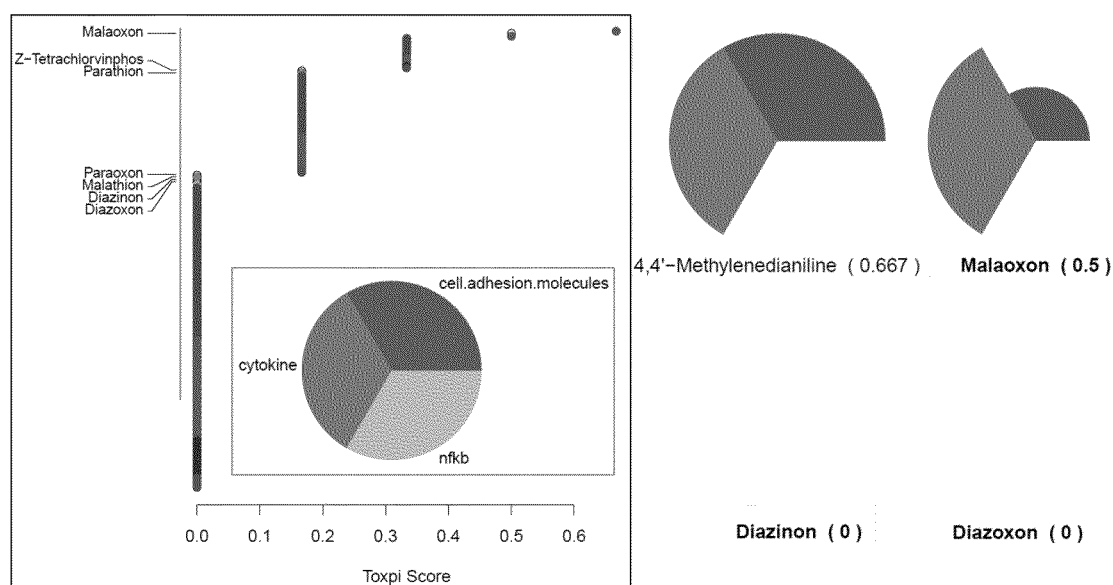

5. "**Oxidative Stressor.**" There were 18 assays mapped to this characteristic in sub-categories of Metalloproteinase (5), Oxidative stress (7), and Oxidative stress marker (6). As it can be observed from the analysis, malathion exhibits intermediate potency based on the results of these in vitro tests, as compared to most potent agents benzo(b)fluoranthene and 4-chloro-1,2-diaminobenzene.
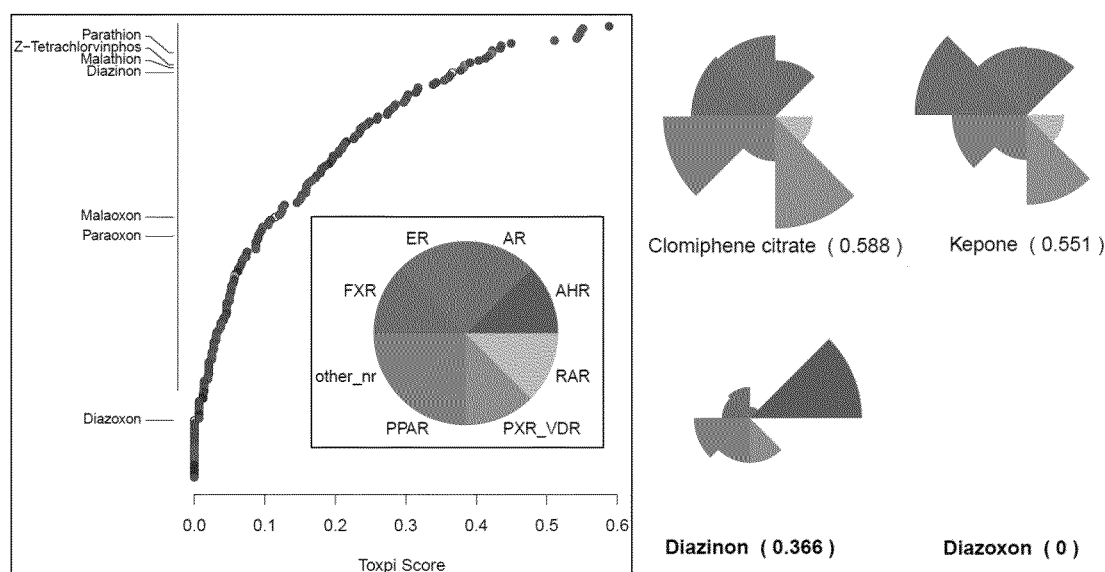

MEETING DRAFT
Do not quote, cite, or distribute

6. "**Induce chronic inflammation**." There were 45 assays mapped to this characteristic in sub-categories of Cell adhesion (14), cytokines (29) and NFkB (2).
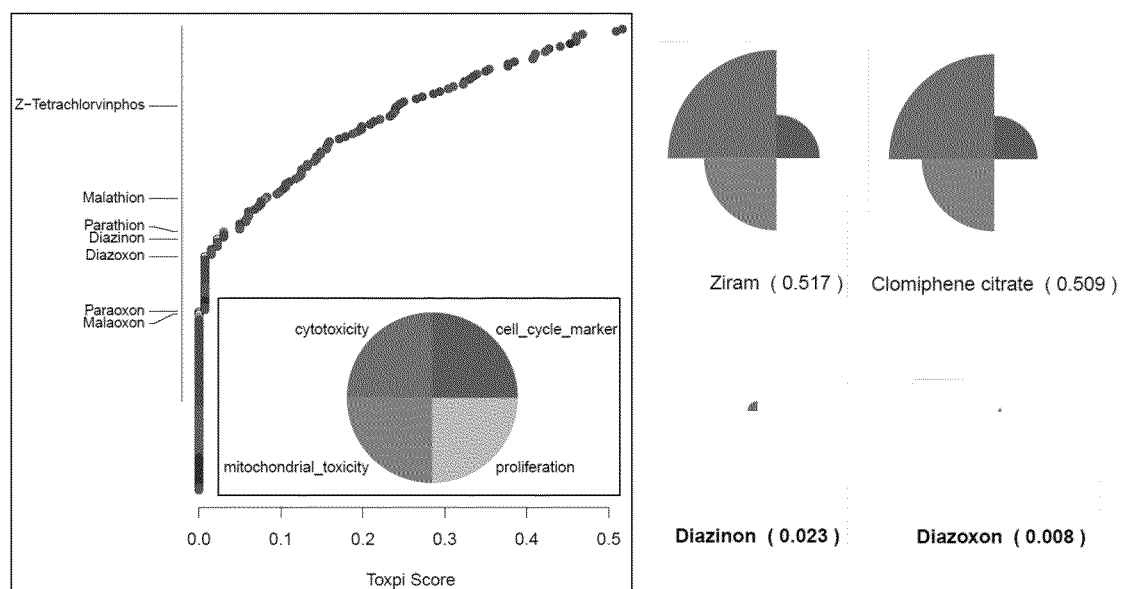

4,4'-Methylenedianiline (0.667)   **Malaoxon (0.5)**

Diazinon (0)   **Diazoxon (0)**

8. "**Modulates receptor-mediated effects**." There were 92 assays mapped to this characteristic in sub-categories of AhR (2), AR (11), ER (18), FXR (7), others (18), PPAR (12), PXR_VDR (7), and RAR (6).

Clomiphene citrate (0.588)   Kepone (0.551)

Diazinon (0.366)   **Diazoxon (0)**

MEETING DRAFT
Do not quote, cite, or distribute

10. "**Alters cell proliferation, cell death and nutrient supply**." There were 68 assays mapped to this characteristic in sub-categories of Cell cycle (16), cytotoxicity (41), mitochondrial toxicity (7) and proliferation (4).