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 of the chemicals included in IARC monograph volume 112 (Tice et al., 2013; PMID 1205784, Kavlock et al 2012 PMID: 22519603). Diazinon, malathion, and parathion, as well as the oxon metabolites, malaoxon and diazoxon, are among the approximately 1000 chemicals tested across the full ToxCast/Tox21 assay battery as of 3 March 2015. This assay battery includes 342 assays, for which data on 821 assay endpoints are publicly available in the US EPA ToxCast Dashboard (www.actor.epa.gov/dashboard). Z-Tetrachlorvinphos (CASRN 22248-79-9; a structural isomer of tetrachlorvinphos) and the oxon metabolite, paraoxon, are among an additional 800 chemicals tested as part of an endocrine profiling effort using a subset of these assays. Glyphosate was not included in either of the chemical libraries.

Detailed information about the chemicals, assays and associated data analysis procedures is also publicly available from (www.epa.gov/toxcast/data). It is of note that while the cell-based assays have a variable degree of metabolic capacity, it is generally limited. [Additionally, the Working Group noted that limited activity of the oxon metabolites in *in vitro* systems may be attributed to high reactivity and short half-life of this compound making interpretation of the results of the in vitro assays difficult.] Detailed information about the chemicals, assays and associated data analysis procedures is also publicly available from (www.epa.gov/toxcast/data).

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 the Monograph volume 112 with respect to their potential impact on mechanisms of carcinogenesis,

the Working Group members first 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 "key characteristics" table). Independent assignments were made by the Working Group members and IARC Monographs staff for each assay type to the one or more "key characteristics". The assignment was based on the biological target being probed by each assay. The consensus assignments comprise 274 assay endpoints that mapped to 7 of the 10 "key characteristics" as shown below.

1) *Is Electrophilic or Can Be Metabolically Activated (31 assay endpoints)*: All assay endpoints measure cytochrome p450 (CYP) inhibition, including aromatase. These assay endpoints are not direct measures of electrophilicity of metabolic activation.

2) *Is Genotoxic (9 assay endpoints):* The only assay endpoints that mapped to this characteristic measure p53 activity. [The Working Group noted that these assays are not direct measures of genotoxicity].

3) *Alters DNA repair or causes genomic instability (0 assay endpoints):* No assay endpoints were mapped to this characteristic.

4) *Induces Epigenetic Alterations (11 assay endpoints):* Assay endpoints mapped to this characteristic measure targets associated with DNA binding and histone modification (e.g., HDAC).

5) *Induces Oxidative Stress (18 assay endpoints):* A diverse collection of assay endpoints measured oxidative stress via cell imaging as well as markers of oxidative stress (e.g., NRF2).

6) *Induces chronic inflammation (45 assay endpoints):* Assay endpoints mapped to this characteristic included inflammatory markers (e.g., IL8 and NFkB activity).

7) *Is Immunosuppressive (0 assay endpoints):* No assay endpoints were mapped to this characteristic.

8) *Modulates receptor-mediated effects (92 assay endpoints):* A large and diverse collection of cell-free and cell-based nuclear and other receptor assays were mapped to this characteristic.

9) *Causes Immortalization (0 assay endpoints):* No assay endpoints were mapped to this characteristic.

10) *Alters cell proliferation/death or nutrient supply (68 assay endpoints):* A collection of assay endpoints measuring cytotoxicity, mitochondrial toxicity, cell cycle and cell proliferation were mapped to this characteristic.

The match of an assay to the "key characteristic" were to provide additional insights into the bioactivity profile of each chemical under evaluation with respect to their potential to interact with, or have an effect on, targets that may be associated with carcinogenesis. In addition, based on the *in vitro* assays that represent each "key characteristic", a comprehensive and unbiased evaluation of the relative activity, as compared to a larger compendium of substances with similar *in vitro* data, may be performed as exemplified by the analysis detailed below.

The Working Group then determined whether a chemical was "active" or "inactive" in each of the selected 274 assay endpoints. Activity calls were determined based on the raw concentration-response data in the ToxCast database using methods published previously (Sipes et al., 2013 PMID: 23611293) and available online (www.epa.gov/toxcast/data).

Next, to integrate the data across individual assay endpoints into the cumulative score for each "key characteristic", the Toxicological Prioritization Index (ToxPi) approach (Reif et al., 2010 PMID: 20826373) and associated software (Reif et al., 2013 PMID: 23202747) were used. In the Working Group's analyses, the ToxPi score provides a measure of the potential for a chemical to be associated with a "key characteristic" relative to the other 182 chemicals that have been

previously evaluated in the IARC monographs that were screened in ToxCast. ToxPi is a dimensionless index score that enables integration of multiple sources of evidence, transformed into visual rankings. 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 and the associated software package (Reif et al., 2013 PMID: 23202747). Within the individual slice, the values are normalized from 0 to 1 based on the range of responses across all chemicals that were included in the analysis.

The list of ToxCast/Tox21 assay endpoints included in the Working Group's analysis, description of each assay endpoint's target and/or model system (e.g., cell type, species, detection technology, etc.), their mapping to 7 of the 10 "key characteristics" of known human carcinogens, and the active/inactive calls for each chemical are available as ***Supplemental Material*** to the Monograph. In addition, the ToxPi software-generated output files for each "key characteristic" are also provided in the supplemental material and can be opened using ToxPi software (Reif et al., 2013 PMID: 23202747) that is freely available for download without a license.

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

Relative effects of tetrachlorvinphos were compared to 180 of a total of XX IARC Monographs-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 as a rank order (left hand side graph in Figures 1-7) of all compounds in the analysis (180 IARC-classified chemicals, 4 IARC Monograph volume 112 compounds and 3 IARC Monograph volume 112 metabolites) arranged in the order of their relative effect. The relative position of Z-tetrachlorvinphos in the ranked list is also shown on the y-axis. The inset in the scatter plot shows the components of the ToxPi chart as sub-categories that comprise assay endpoints 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. Because Z-tetrachlorvinphos was not tested against many of the assay endpoints for some of the characteristics discussed below, the ToxPi chart of Z-tetrachlorvinphos is shown below only for the Modulates receptor-mediated effects key characteristic where the majority of assay endpoints (>90%) were tested with this chemical.

#1. "Electrophilic or ability to undergo metabolic activation." Z-tetrachlorvinphos was tested only in the 2 aromatase inhibition assay endpoints demonstrating activity in a one cell-based, but not in a cell-free inhibition assay endpoint. Z- tetrachlorvinphos was not tested in any of the other 29 cell-free CYP inhibition assay endpoints.

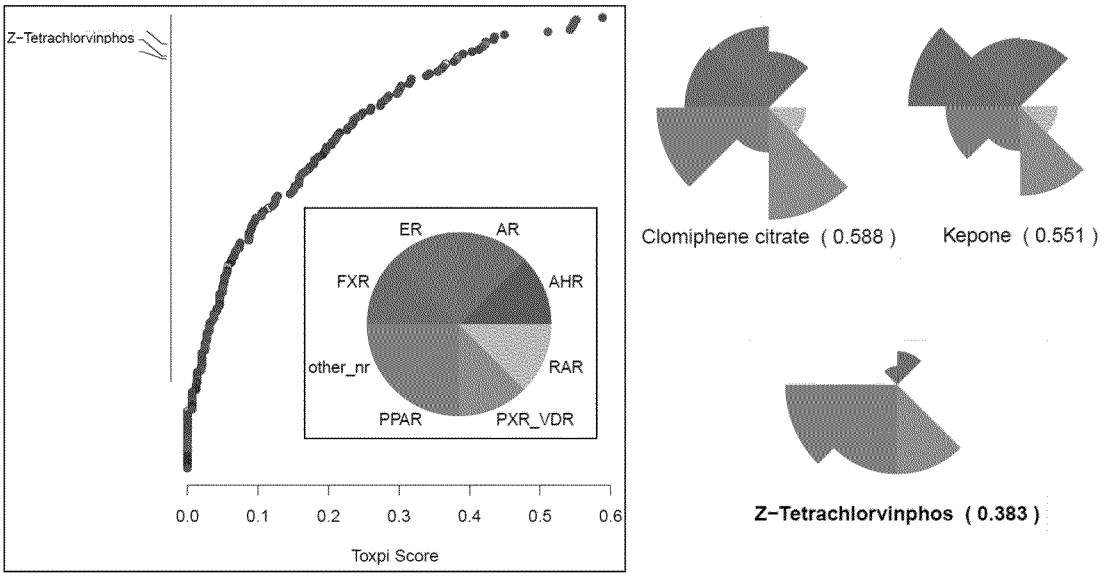
#2. "Genotoxic." Z-tetrachlorvinphos was tested in 6 of the 9 assay endpoints showing activity in 2 assay endpoints. In comparison, the most active chemical in the dataset, chlorobenzilate, showed activity in 7 out of the 8 assay endpoints for which it was tested. Z-tetrachlorvinphos activated the ATG_p53_CIS_up assay endpoint (see ***Supplemental Materials*** for additional details on each assay), an assay shown to be also associated with oxidative stress (Martin et al. 2010 PMID: 20143881).

#4. "Epigenetic alterations." Z-tetrachlorvinphos was active in all 4 of the DNA binding assay endpoints, but was not tested in any of the 7 transformation assay endpoints. [The Working Group notes that, similar to the activity in the p53 assay endpoints, the positive response in the ATG_CIS assay endpoints is most likely due to the activation of oxidative stress].

#5. "Oxidative Stressor." Z-tetrachlorvinphos was tested in all 6 oxidative stress marker assay endpoints and exhibited intermediate activity based on being active in 3 out of 6. A comparison to the most active chemicals, carbaryl and tannic acid, is limited due to the incomplete testing of Z-tetrachlorvinphos. In particular, Z- tetrachlorvinphos was not tested in any of the metalloproteinase or oxidative stress assay endpoints. Therefore, its potential to act as an oxidative stressor has not been evaluated beyond activation of other oxidative stress markers (e.g., NRF2, Metal Response Element), as well as activity in an assay targeting the antioxidant response element (i.e., Tox21_ARE_BLA_agonist).

#6. "Induces chronic inflammation." It is notable that Z-tetrachlorvinphos was active in 1 of the 2 NF-kB assay endpoints for which only 7 of the 185 chemicals in the analysis were active. Z-tetrachlorvinphos was not tested in the panel of 14 and 29 assay endpoints that comprise the cytokine and cell adhesion molecule assay groupings, respectively.

8. "Modulates receptor-mediated effects." Z-tetrachlorvinphos was tested in 89 of the 92 assay endpoints mapped to this characteristic (comprising assays for aryl hydrocarbon receptor (AhR, 2 assay endpoints), androstane receptor (AR, 11), estrogen receptor (ER, 18), farnesoid X receptor (FXR, 7), other orphan nuclear receptors (18), peroxisome proliferator activated receptors (PPAR, 12), pregnane X receptor/vitamin D receptor (PXR_VDR, 7), and retinoic acid receptor (RXR, 6)). As compared to other chemicals evaluated by the IARC Monographs, it demonstrated appreciable capacity to interact with nuclear and other receptors and, similar to the top 2 ranking chemicals (clomiphene citrate and kepone), broad specificity nuclear receptor targets was observed. Z-tetrachlorvinphos showed consistent PXR activation and activity in assay endpoints representative of antagonists of PPAR and other nuclear receptors. Z-tetrachlorvinphos activated 2 of the 18 ER assay endpoints (ATG_ERa_TRANS_up and ATG_ERE_CIS_up). Of the 11 AR assay endpoints, 2 assay endpoints were run in an antagonist mode and Z-tetrachlorvinphos was active in both and in 1 of 2 protein complementation assay endpoints that test for agonist and antagonist activity.



#10. "Alters cell proliferation, cell death and nutrient supply." Z-tetrachlorvinphos was tested in 27 out of the 68 assay endpoints. There were 68 assay endpoints mapped to this characteristic in sub-categories of cell cycle (16), cytotoxicity (41), mitochondrial toxicity (7) and proliferation (4). Z-tetrachlorvinphos showed moderate impact on the assay endpoints in this group as compared to the top 2 ranking chemicals, clomiphene citrate and ziram. Z-tetrachlorvinphos was active in the only mitochondrial toxicity assay it was tested in, Tox21_Mitochondrial_Toxicity.

Overall, Z-tetrachlorvinphos was active in 36 of the 137 assay endpoints for which it was tested. The results of ToxPi analysis of the ToxCast/Tox21 data for Z-tetrachlorvinphos supports findings in other model systems as described in section 4.2. These include aromatase inhibition, multiple nuclear receptor activities, oxidative stress and some cytotoxic effects.