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 environmental and other chemicals (Tice et al., 2013; PMID 1205784, Kavlock et al 2012 PMID: 22519603). As of 3 March 2015, data on 821 assay endpoints derived from 342 assays are publicly available in the US EPA ToxCast Dashboard (www.actor.epa.gov/dashboard). 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.

More than 30 organophosphate pesticides or their oxon metabolites, including diazinon, malathion, parathion, and as well as oxon metabolites malaoxon and diazoxon are among the approximately 1000 chemicals tested across the full assay battery as of 3 March 2015, the ToxCast program has, comprising more than 800 *in vitro* tests,. An additional 800 chemicals, including z-tetrachlorvinphos (CASRN 22248-79-9; a structural isomer of tetrachlorvinphos) and the oxon metabolite paraoxon, were tested as part of an endocrine profiling effort using a subset of these assays. Glyphosate was not included in either of the chemical libraries.

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 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 "key characteristics" table). Independent assignments were made

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by the Working Group members and IARC Monographs staff 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

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 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.

To perform the analysis described in this section, it was determined whether a chemical was "active" or "inactive" in each of the selected 274 assays. Activity calls were determined based on

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the raw concentration-response data in the ToxCast database using methods published previously (Sipes et al., 2013 PMID: 23611293).

Next, to integrate the data across individual assays 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) was used. In the analyses included in this section, the ToxPi score provides a relative measure of the potential for a chemical to be associated with a "key characteristic" relative to the other 182 chemicals of the 950? that have been previously evaluated in the IARC monographs that were included in the list of compounds screened in ToxCast/Tox21. 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 assays included in this analysis, description of each assay's target and/or model system (e.g., cell type, species, reporter plasmid sequence, etc.), their mapping to 7 "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.
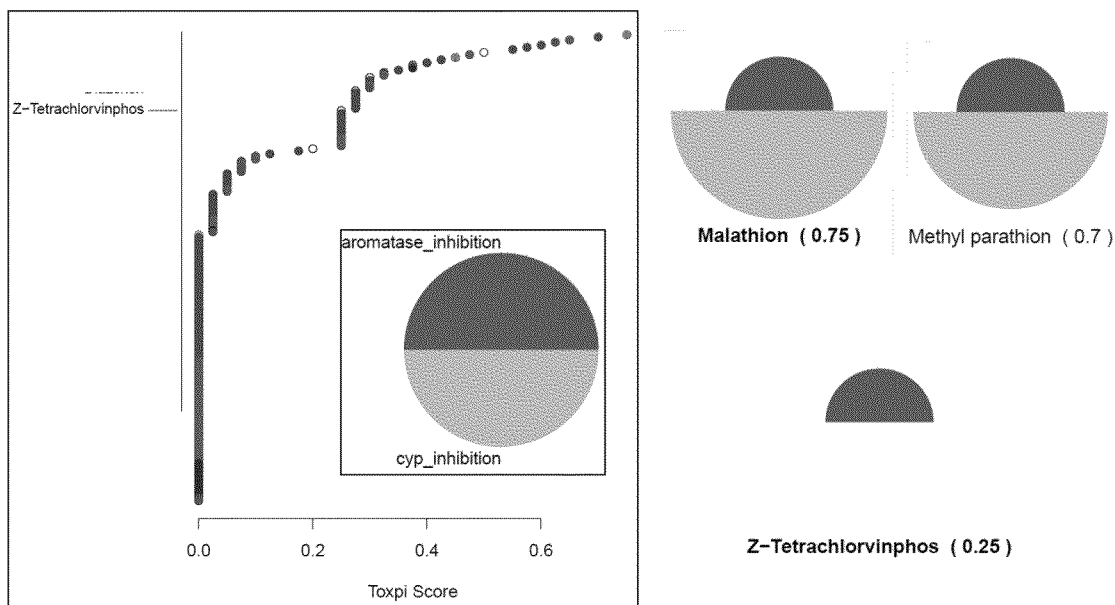

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4.3.3. Tetrachlorvinphos-specific effects across 7 "key characteristics" based on *in vitro* screening data.

Relative effects of tetrachlorvinphos were compared to 182? 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 1-7) of all compounds in the analysis (180 IARC-classified chemicals, 4 Monograph 112 compounds and 3 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 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 Z-tetrachlorvinphos are shown below.
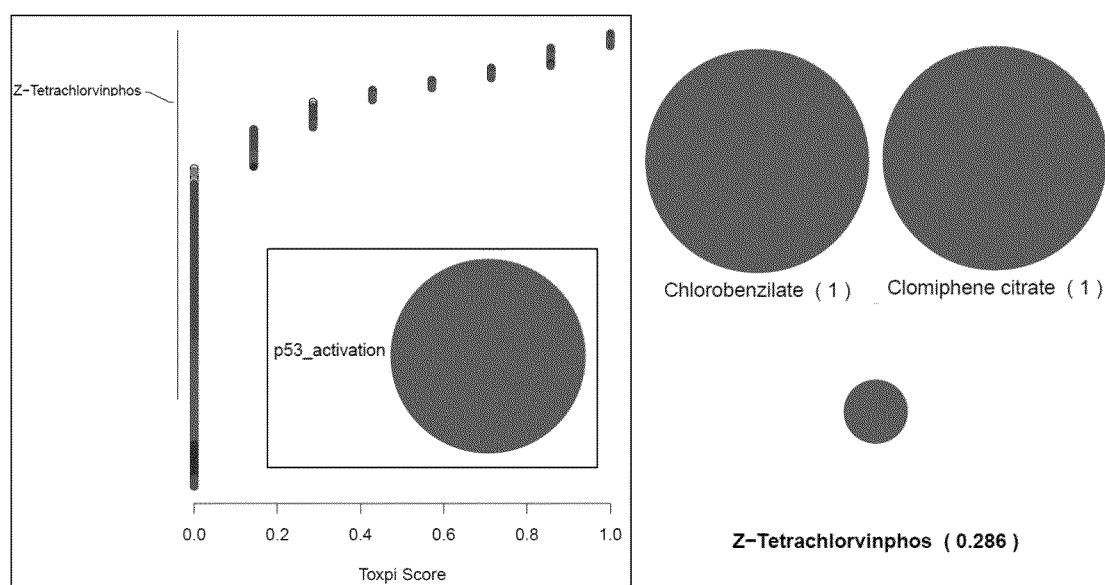
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#1. "Electrophilic or ability to undergo metabolic activation." There were 31 assays mapped to this characteristic in sub-categories of CYP inhibition (29) and aromatase inhibition (2). Z-tetrachlorvinphos was tested only in the 2 aromatase inhibition assays demonstrating activity in a cell-based, but not in a cell-free inhibition assay. Z-tetrachlorvinphos was not tested in any of the other 29 cell-free CYP inhibition assays.
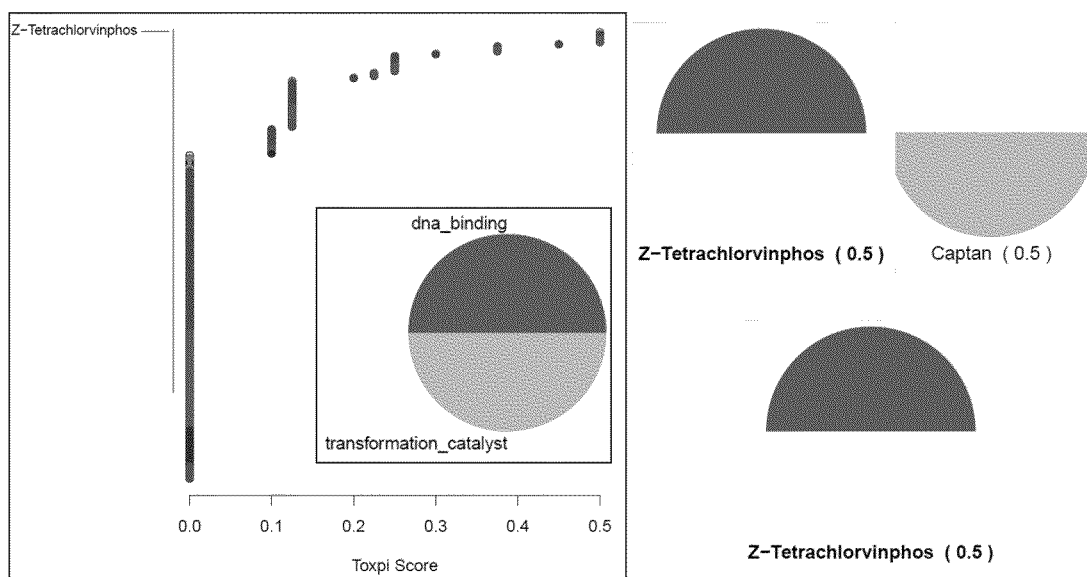

Malathion (0.75)   Methyl parathion (0.7)

Z-Tetrachlorvinphos (0.25)

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#2. "Genotoxic." There were 9 assay mapped to this characteristic all belonging to a sub-category of p53 activation. Z-tetrachlorvinphos was tested in 6 of the 9 assays showing activity in 2 assays as compared to chlorobenzilate, which showed activity in 7 out of the 8 assays 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).
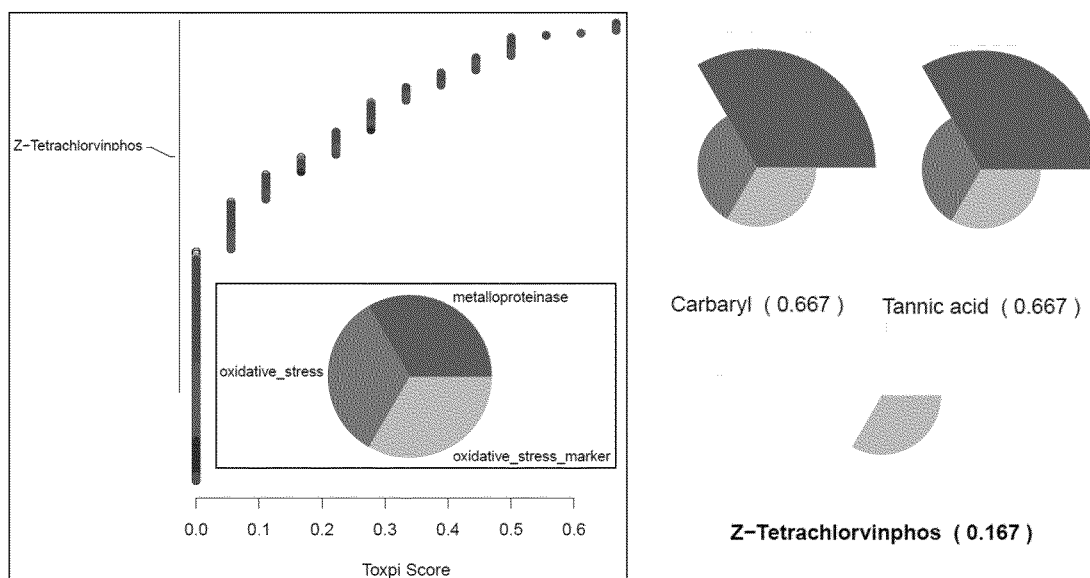
#4. "Epigenetic alterations." There were 11 assays mapped to this characteristic in sub-categories of DNA binding (4) and transformation (7). Z-tetrachlorvinphos was active in all 4 of the DNA binding assay endpoints, but was not tested in any of the 7 transformation assays. Similar to the activity in the p53 assays, the positive response in the ATG_CIS assays is most likely due to the activation of oxidative stress.
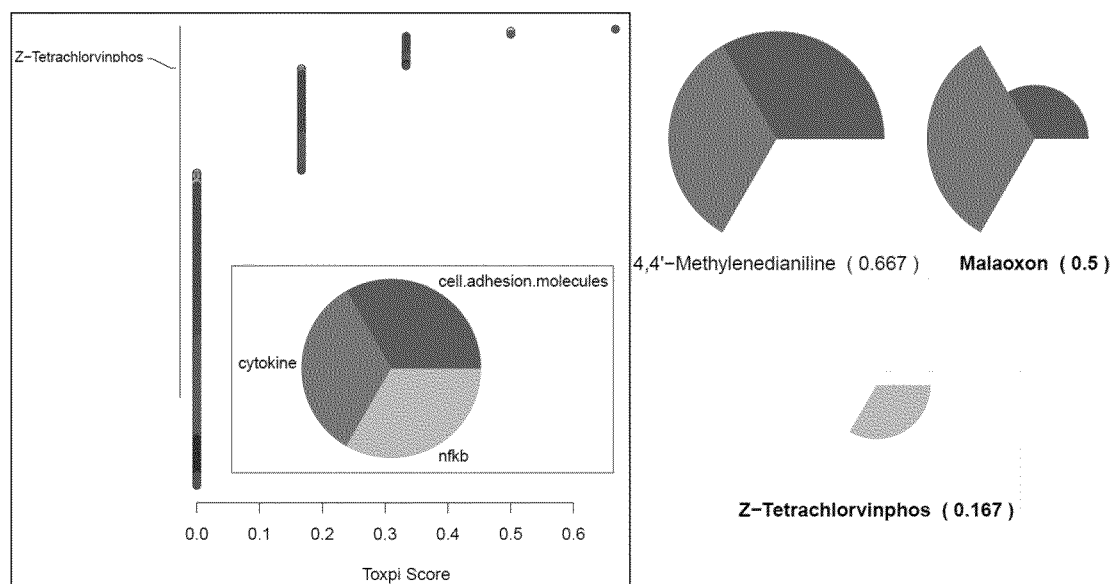

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#5. "Oxidative Stressor." There were 18 assays mapped to this characteristic in sub-categories of Metalloproteinase (5), Oxidative stress (7), and Oxidative stress marker (6). As it can be observed from the analysis, Z-tetrachlorvinphos exhibits intermediate activity based on the results of these in vitro tests, as compared to most active chemicals, carbaryl and tannic acid. Z- tetrachlorvinphos was not tested in any of the metalloproteinase (red slice) or oxidative stress (gray slice) assays; therefore, its full potential to act as an oxidative stressor has not been evaluated beyond activation of other (yellow slice) 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).
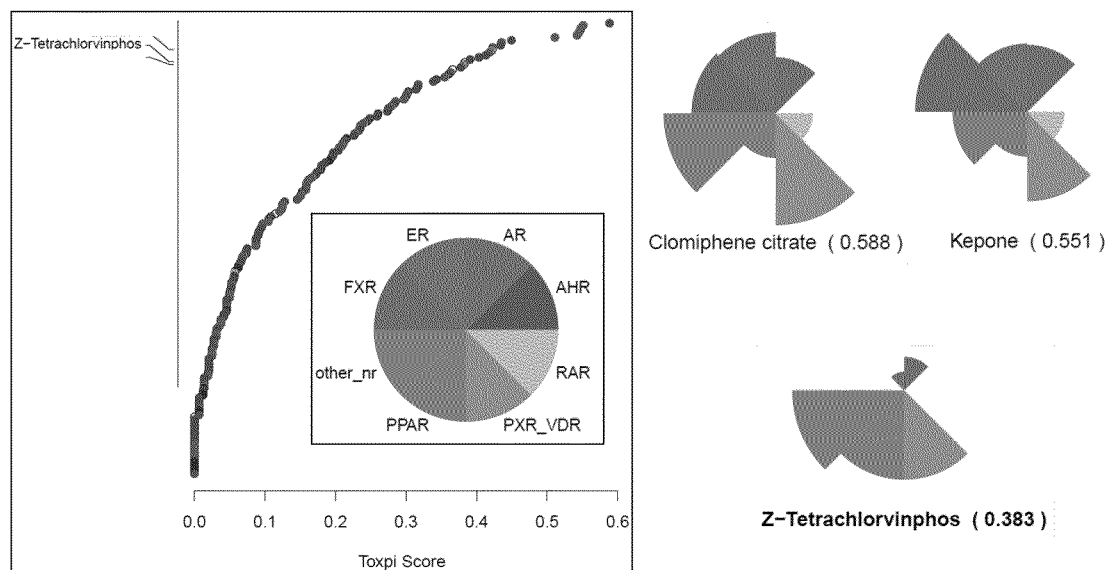

Carbaryl (0.667)   Tannic acid (0.667)

Z–Tetrachlorvinphos (0.167)

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6. "Induces chronic inflammation." There were 45 assays mapped to this characteristic in sub-categories of cell adhesion (14), cytokines (29) and NFkB (2). Z-tetrachlorvinphos was not tested in the panel of assays which comprised the cytokine (gray slice) and cell adhesion molecule (red slice) assay groupings. It is notable that Z-TCVP was active in 1 of 2 NF-kB assays for which only 7 of the 185 chemicals in the analysis were active.
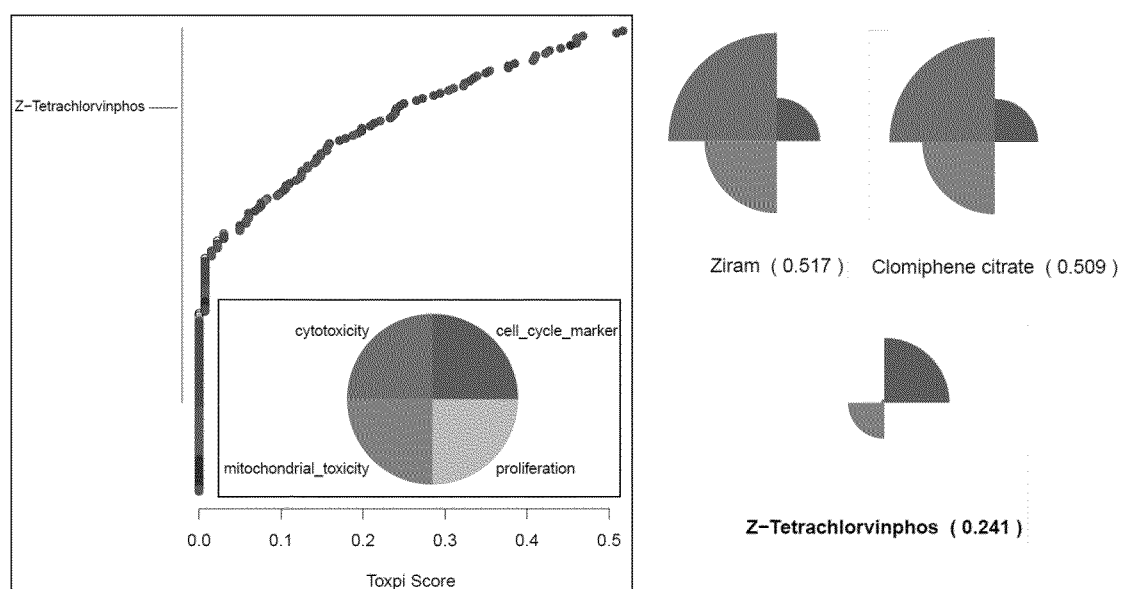

4,4'–Methylenedianiline (0.667)   **Malaoxon (0.5)**

Z–Tetrachlorvinphos (0.167)

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8. "Modulates receptor-mediated effects." There were 92 assays mapped to this characteristic in sub-categories of aryl hydrocarbon receptor (AhR, 2 assays), androstane receptor (AR, 11), estrogen receptor (ER, 18), farnesoid X receptor (FXR, 7), other orphan nuclear receptorss (18), peroxisome proliferator activated receptors (PPAR, 12), pregnane X receptor/vitamin D receptor (PXR_VDR, 7), and retinoic acid receptor (RXR, 6). Z-tetrachlorvinphos was tested in 89 of the 92 assays. As compared to other IARC chemicals, 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 assays representative of antagonists of PPAR and other nuclear receptors. Z-tetrachlorvinphos activated 2 of the 18 ER assays (ATG_ERa_TRANS_up and ATG_ERE_CIS_up). Of the 11 AR assays, 2 assays were run in an antagonist mode and Z-tetrachlorvinphos was active in both and in 1 of 2 protein complementation assays that test for agonist and antagonist activity.

Clomiphene citrate (0.588)   Kepone (0.551)

Z–Tetrachlorvinphos (0.383)

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#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). Z-tetrachlorvinphos was tested in 27 out of the 68 assays. Z-tetrachlorvinphos showed moderate impact on the assays 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.

Ziram (0.517)   Clomiphene citrate (0.509)

Z-Tetrachlorvinphos (0.241)

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 corraborates findings in other model systems as described in section 4.2. These include aromatase inhibition, promiscuous nuclear receptor activity, oxidative stress and some cytotoxic effects.

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