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, including diazinon, malathion, parathion, and as well as oxon metabolites, malaoxon and diazoxon, are among the approximately 1000 chemicals tested across the full ToxCast/Tox21 assay battery as of 3 March 2015. 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. [The 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.]

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 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 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):* All assay endpoints mapped to this characteristic measure p53 activity. 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.

To perform the analysis described in this section, it was 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 analyses included in this section, 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

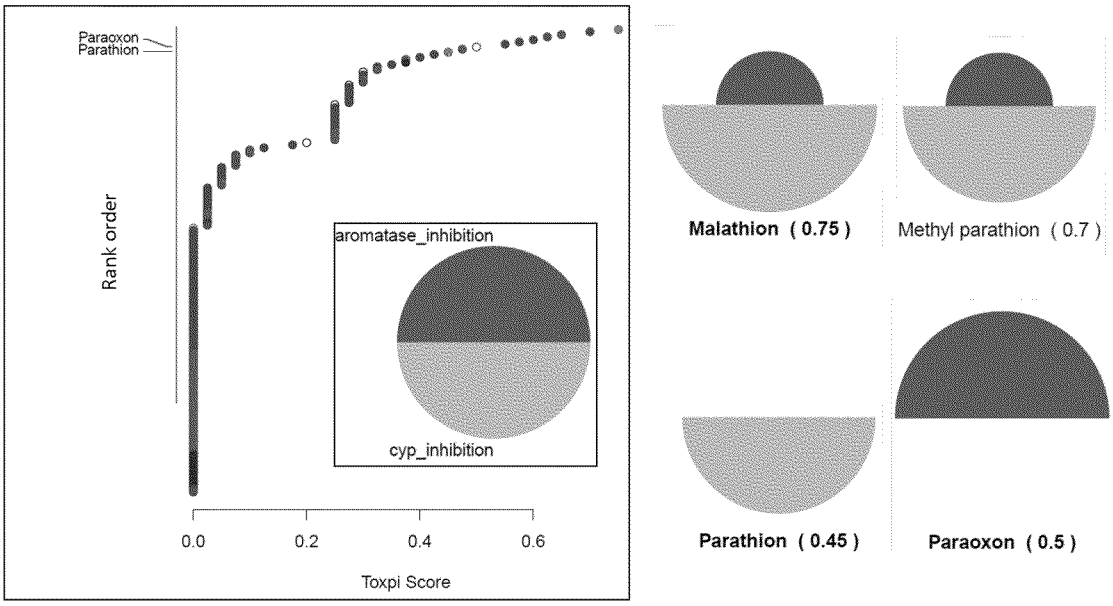
dimensionless index score that enables integration of multiple sources of evidence, transformed into visual rankings. Different data are translated into ToxPi scores to derive slice-wise scores for all compounds as detailed below and in the publications describing the approach and the associated software 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 this 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.

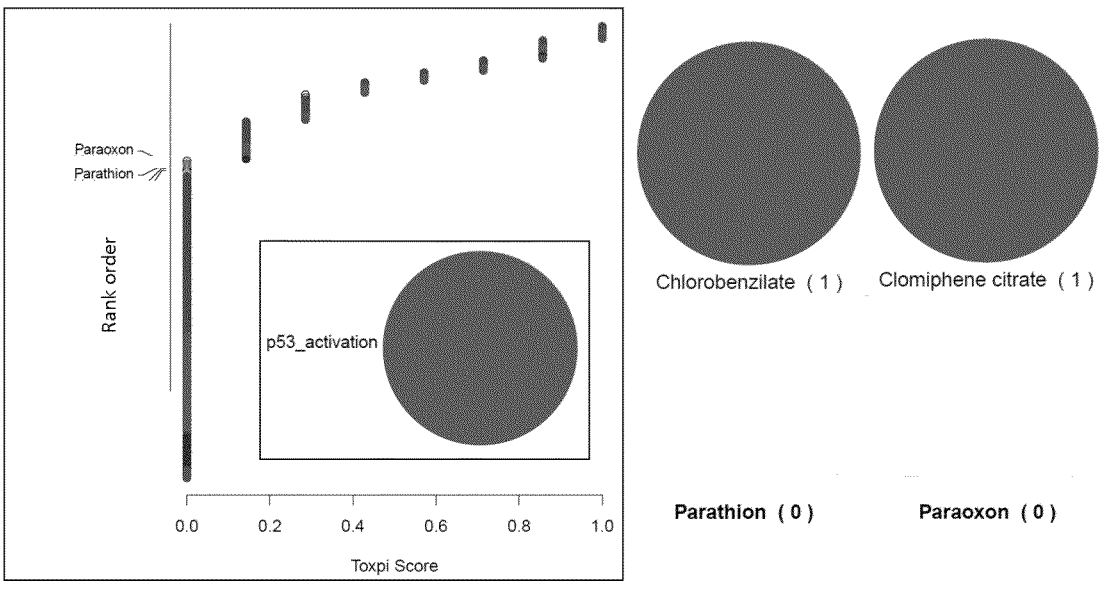
4.3.3. Parathion-specific effects across 7 of the 10 "key characteristics" based on *in vitro* screening data.

Relative effects of parathion were compared to 180 of a total of XX IARC Monograph-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 of all compounds in the analysis (180 IARC-classified chemicals, 4 Monograph 112 compounds and 3 metabolites) arranged in the order of their relative activity. The relative position of parathion and paraoxon 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 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. The ToxPi charts of parathion and paraoxon are shown below where the majority of assay endpoints (>90%) were tested for the key characteristic.

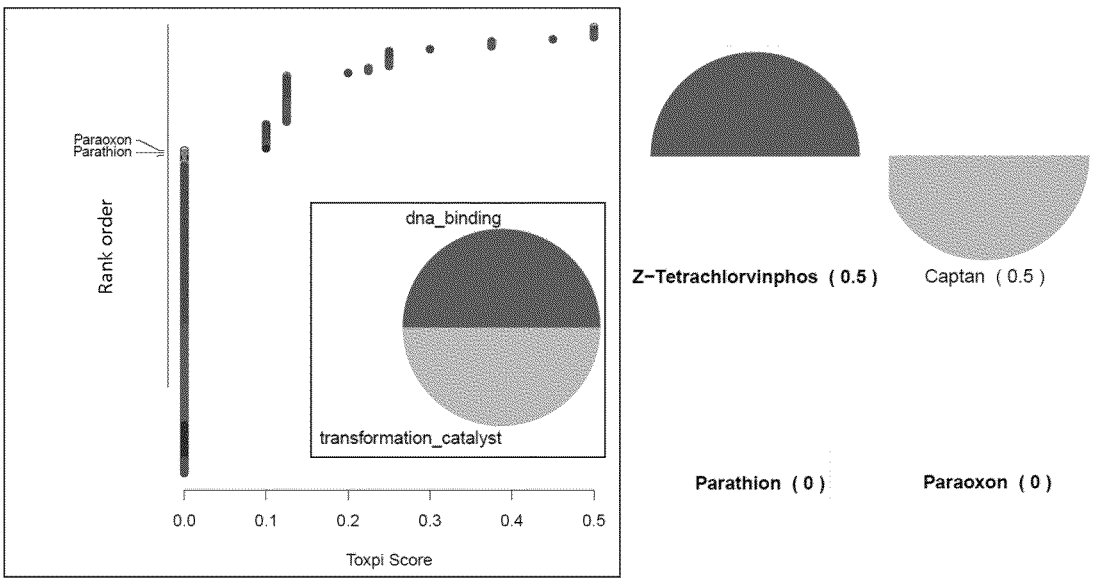
#1. "**Electrophilic or ability to undergo metabolic activation**." Parathion was tested in all 29 CYP inhibition assay endpoints and both aromatase inhibition assay endpoints. Parathion was active in 18 of the 29 CYP inhibition assay endpoints. In comparison, the top-ranked chemical malathion was active in 20 out of 29 assay endpoints. All 29 CYP inhibition assay endpoints are cell-free. Parathion was tested in 2 aromatase inhibition assay endpoints but found to be inactive. Paraoxon was only tested in the 2 aromatase inhibition assay endpoints and was active in both.



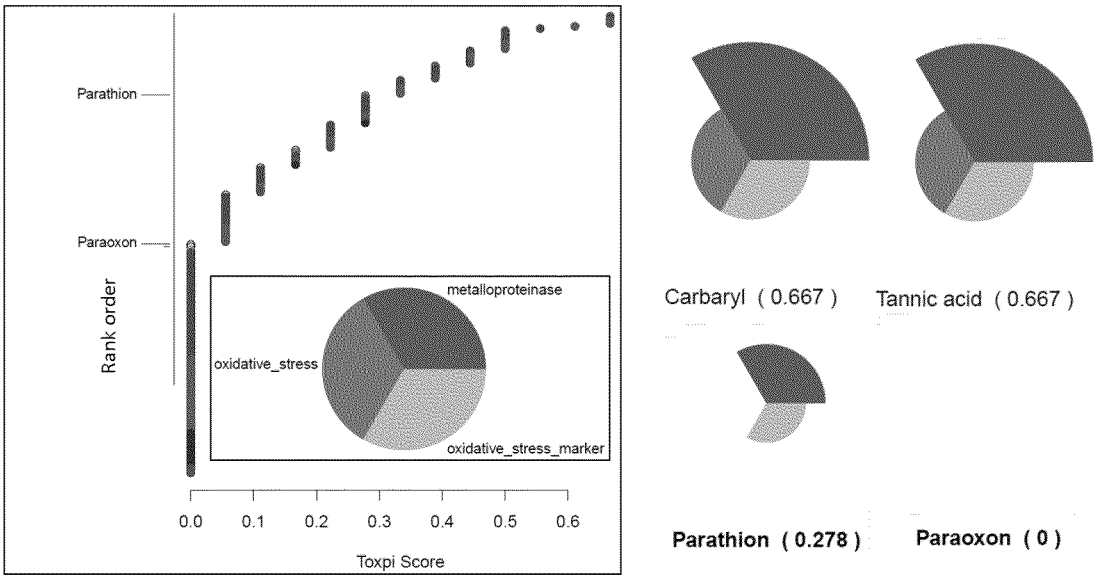
#2. "**Genotoxic**." Parathion and paraoxon were inactive in all of the 9 and 6, respectively, of the 9 available p53 assay endpoints. In comparison, chlorobenzilate showed activity in 7 out of the 8 assay endpoints for which it was tested.



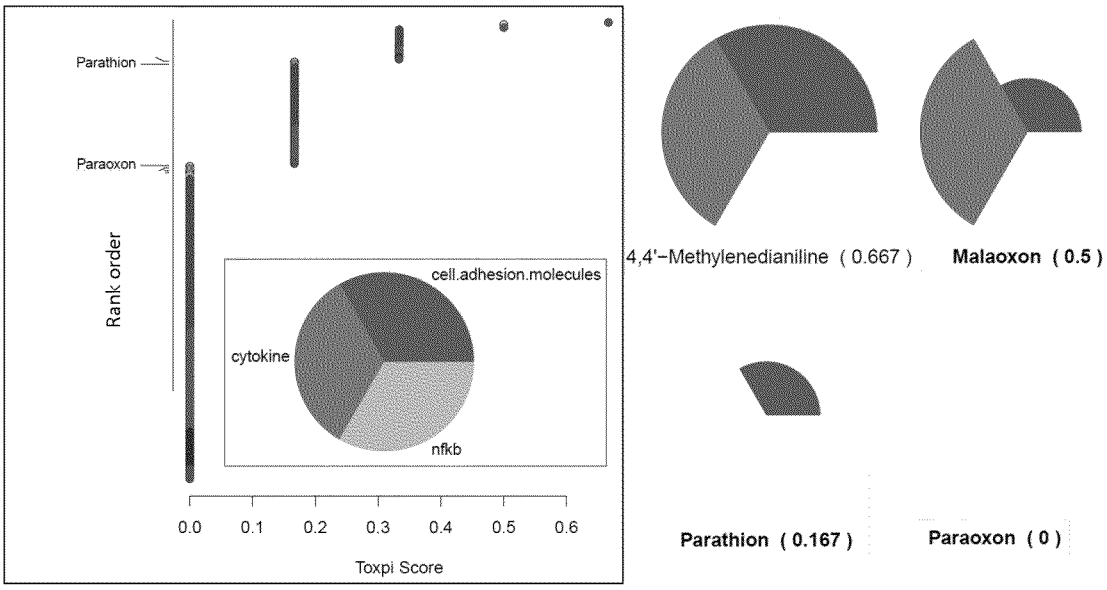
#4. "**Epigenetic alterations**." Parathion and paraoxon were inactive in 11 and 4, respectively, of the 11 tested assay endpoints associated with epigenetic alterations. In comparison, Z-tetrachlorvinphos was active in all 4 of the DNA binding assay endpoints, but was not tested in any of the 7 transformation assay endpoints.



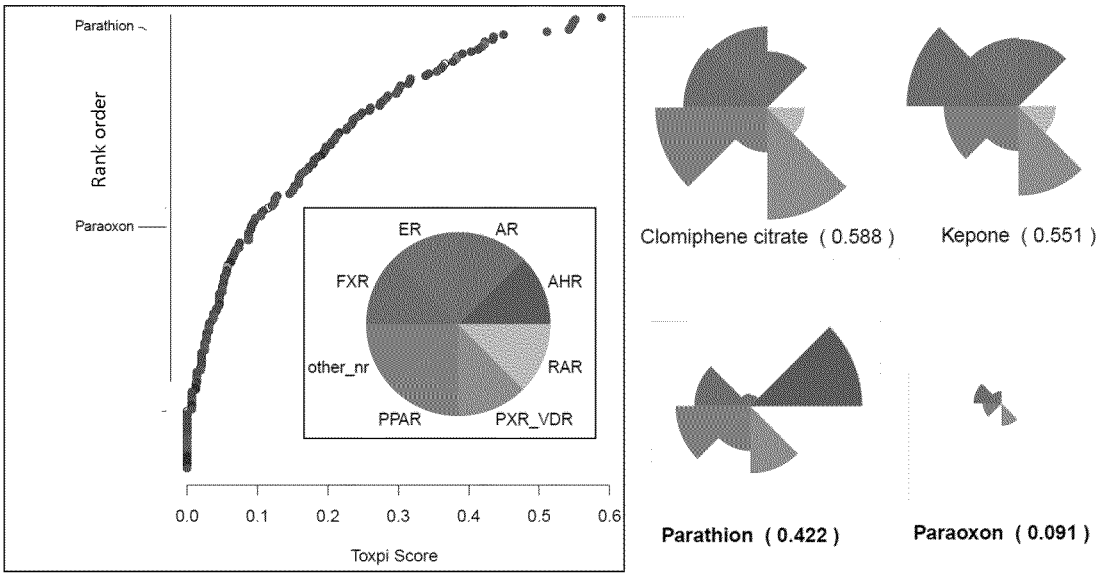
#5. "**Oxidative Stressor.**" Parathion was tested in all 18 assays and active in 2 out of the 6 oxidative stress marker assay endpoints. Paraoxon was inactive across the 7 assay endpoints for which it was tested. In comparison to the top 2 chemicals, carbaryl and tannic acid, parathion was moderately active across the metalloproteinases and oxidative stress markers. The metalloproteinase assay endpoints were highly selective with the maximal responder (i.e., carbaryl) only activating 2 out of 5 assay endpoints. Parathion displayed activity in a single assay, BSK_hDFCGF_MMP1_up. Parathion also induced transcription factor activation of NFR2 and the metal response element (MRE)..



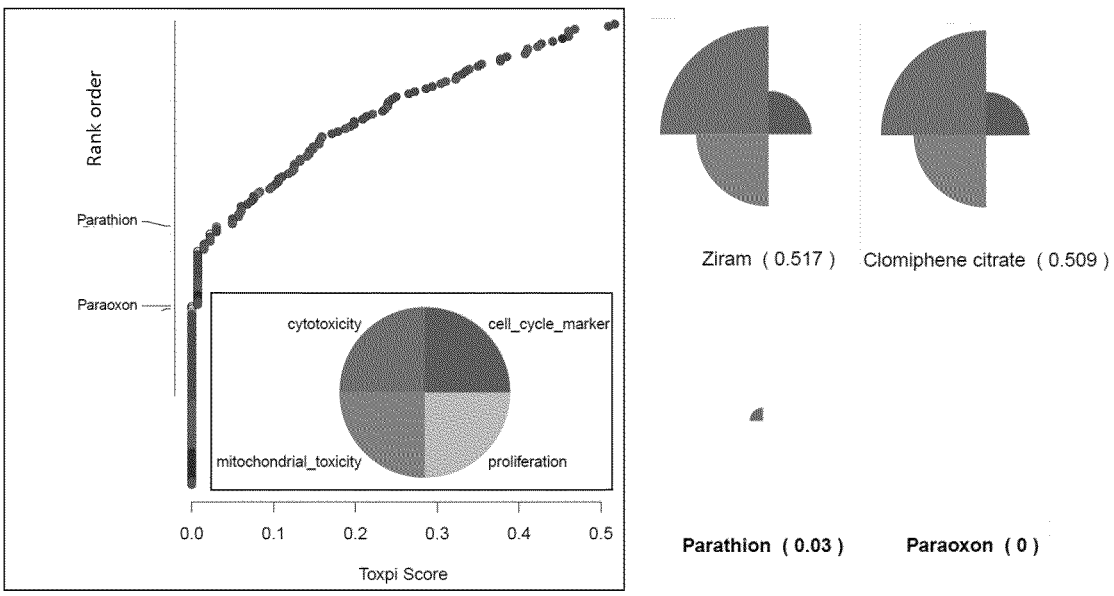
#6. "**Induce chronic inflammation**." Parathion and paraoxon were tested in 45 and 2 (both NFkB), respectively, of the 45 assay endpoints and showed weak to no activity across assay endpoints associated with chronic inflammation as compared to the top ranked compounds. The 45 assay endpoints were mapped to this characteristic in sub-categories of cell adhesion (14), cytokines (29) and NFkB (2).



8. "**Modulates receptor-mediated effects**." Parathion and paraoxon were tested in 92 and 89, respectively, of the 92 assay endpoints in this group. As shown in comparison to top-scoring chemicals clomiphene citrate and kepone, parathion selectively activated both AHR assay endpoints, ATG_Ahr_CIS_up and Tox21_AhR. In addition, parathion showed appreciable activity in 14 of "other nuclear receptor" assay endpoints, making it one of the higher ranked chemicals overall. Paraoxon showed relatively weak receptor activity. The 92 assay endpoints were 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).



#10. "**Alters cell proliferation, cell death and nutrient supply**." Parathion and paraoxon were tested in 67 and 27, respectively, of the 68 assay endpoints, but showed almost no activity in assay endpoints associated with cytotoxicity or cellular proliferation. The 68 assay endpoints were mapped to this characteristic in sub-categories of cell cycle (16), cytotoxicity (41), mitochondrial toxicity (7) and proliferation (4).



Overall, parathion was active in 42 of 273 assay endpoints for which it was tested. The results of ToxPi analysis of the ToxCast/Tox21 data for parathion corraborate findings in other model systems as described in section 4.2. These include AHR activity, strong CYP inhibition, weak non-specific receptor activity, and moderate oxidative stress activity. Its oxon metabolite, paraoxon, showed little bioactivity under the conditions of these assay endpoints with only 7 actives across 134 tested assay endpoints. The limited activity of paraoxon may be attributed to high reactivity and short half-life of this compound making interpretation of the results of the *in vitro* assay endpoints difficult.