

Use of high throughput screening data in IARC monograph evaluations

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Conflict of Interest Statement

- The author declares no relevant conflicts of interest with respect to the content of this presentation
- This presentation does not reflect the official views of WHO, IARC, U.S. EPA, or any other organization or third party, and contains personal opinions of the authors

Background: Role of Mechanistic Data in IARC Monographs

Step 1: Categorize each line of evidence using defined terms

Cancer in humans

- *Sufficient evidence*
- *Limited evidence*
- *Inadequate evidence*

Cancer in experimental animals

- *Sufficient evidence*
- *Limited evidence*
- *Inadequate evidence*

Mechanistic and other relevant data

- “Weak,” “moderate,” or “strong” evidence?
- Does this– or can it– occur in humans?

Role of mechanistic evidence:

- Evaluated separately from human and experimental animal evidence.
- Can lead to “upgrade” or “downgrade” in absence of sufficient human evidence.
- Evaluated using “key characteristics” described by Dr. Smith.

Step 2: Integrate findings in overall evaluations

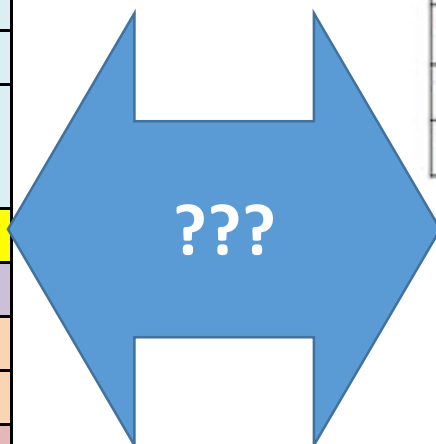
Overall evaluation

- Group 1 *Carcinogenic to humans*
- Group 2A *Probably carcinogenic to humans*
- Group 2B *Possibly carcinogenic to humans*
- Group 3 *Not classifiable as to its carcinogenicity to humans*

Background: Key characteristics and High throughput screening

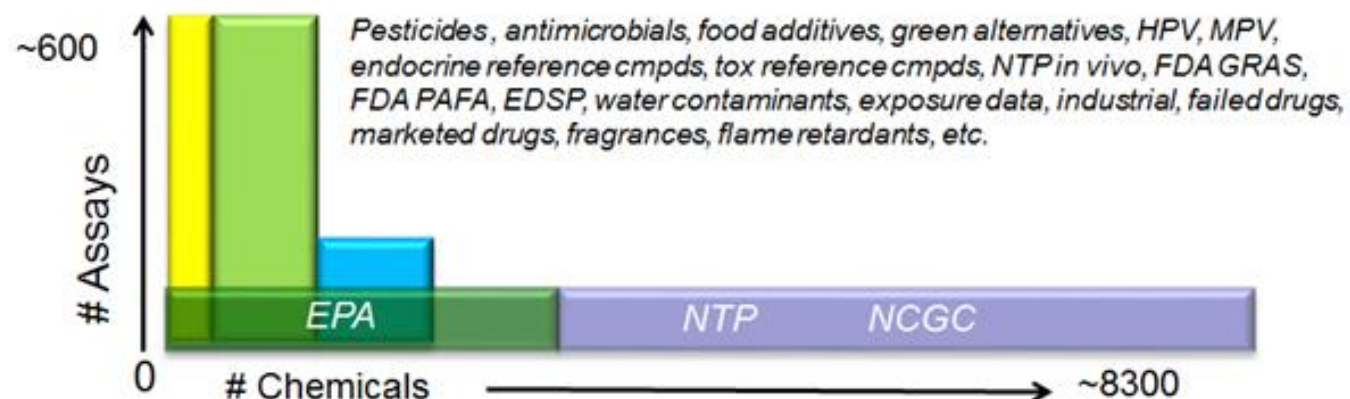
Key characteristics of carcinogens identified by IARC expert review of all Group1 agents (Smith et al., 2015)

Characteristic ¹
1. Is Electrophilic or Can Be Metabolically Activated
2. Is Genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces Epigenetic Alterations
5. Induces Oxidative Stress
6. Induces chronic inflammation
7. Is Immunosuppressive
8. Modulates receptor-mediated effects
9. Causes Immortalization
10. Alters cell proliferation, cell death or nutrient supply



Listing of the Chemical x Assay dimensions and time-line for completion of the various phases of EPA's ToxCast and Tox21 testing programs

Inventory	Chemicals	Assays	Endpoints	Completion	Available
ToxCast Phase I	293	~600	~700	2011	Now
ToxCast Phase II	767	~600	~700	03/2013	Now
ToxCast E1K	800	~50	~120	03/2013	Now
Tox21-EPA	3726	>80	>150	Ongoing	Ongoing
Tox21-Total	~8300	>80	>150	Ongoing	Ongoing



Recent Case Studies from IARC monographs

	Case Study 1	Case Study 2	Case Study 3
Monograph:	110	112	113
Chemical(s):	PFOA	Malathion, Z-Tetrachlorvinphos, Parathion, Diazinon	DDT, 2,4-D, Lindane
Background and Motivation:	It has been hypothesized that that rodent tumors caused by PFOA are not relevant to humans because they act predominantly through PPAR activation.	Available mechanistic data have gaps due to uneven resources devoted to different mechanisms. Differences in experimental design impair the ability to compare data across chemicals.	

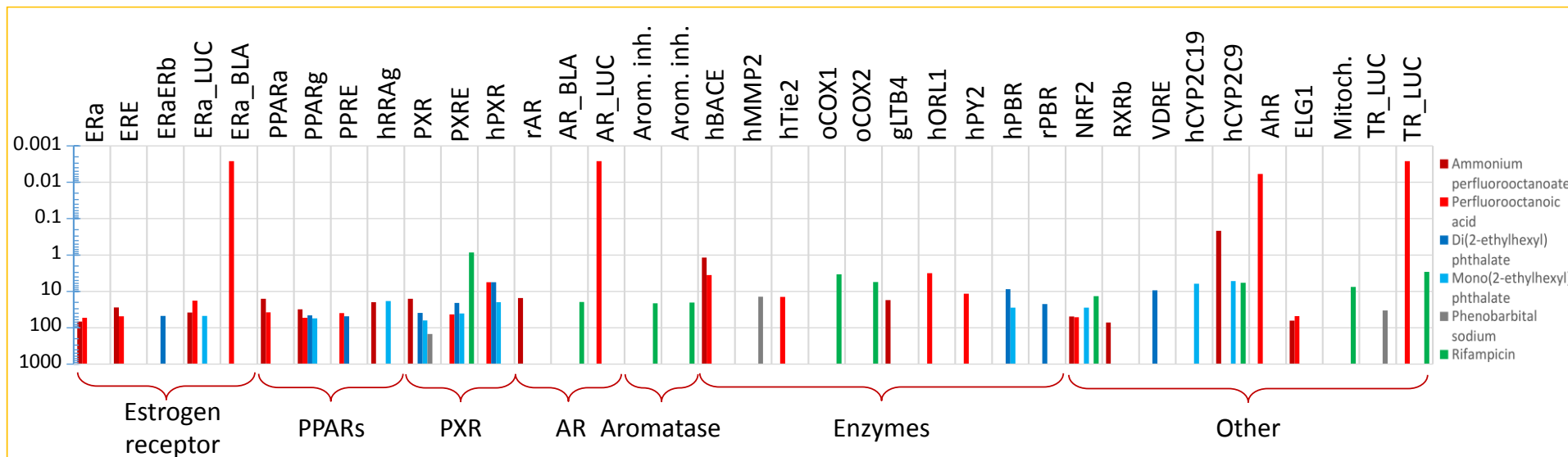
Case Study 1: PFOA (Vol 110)

Mechanistic Question:

- Does PFOA act through activation of nuclear receptors? If yes, does it exclusively activate PPAR family of the receptors?

Approach:

A comparative analysis of *in vitro* screening results of PFOA with those of several prototypical nuclear receptor activating compounds



Comparison of ToxCast AC₅₀s (in microM) for nuclear-receptor-related assays between PFOA (including its ammonium salt) and selected “prototypical” compounds DEHP (including its metabolite MEHP), Phenobarbital, and Rifampicin. Longer bar indicates greater potency (lower AC₅₀). Assays for which all compounds were negative are not displayed.

Conclusion:

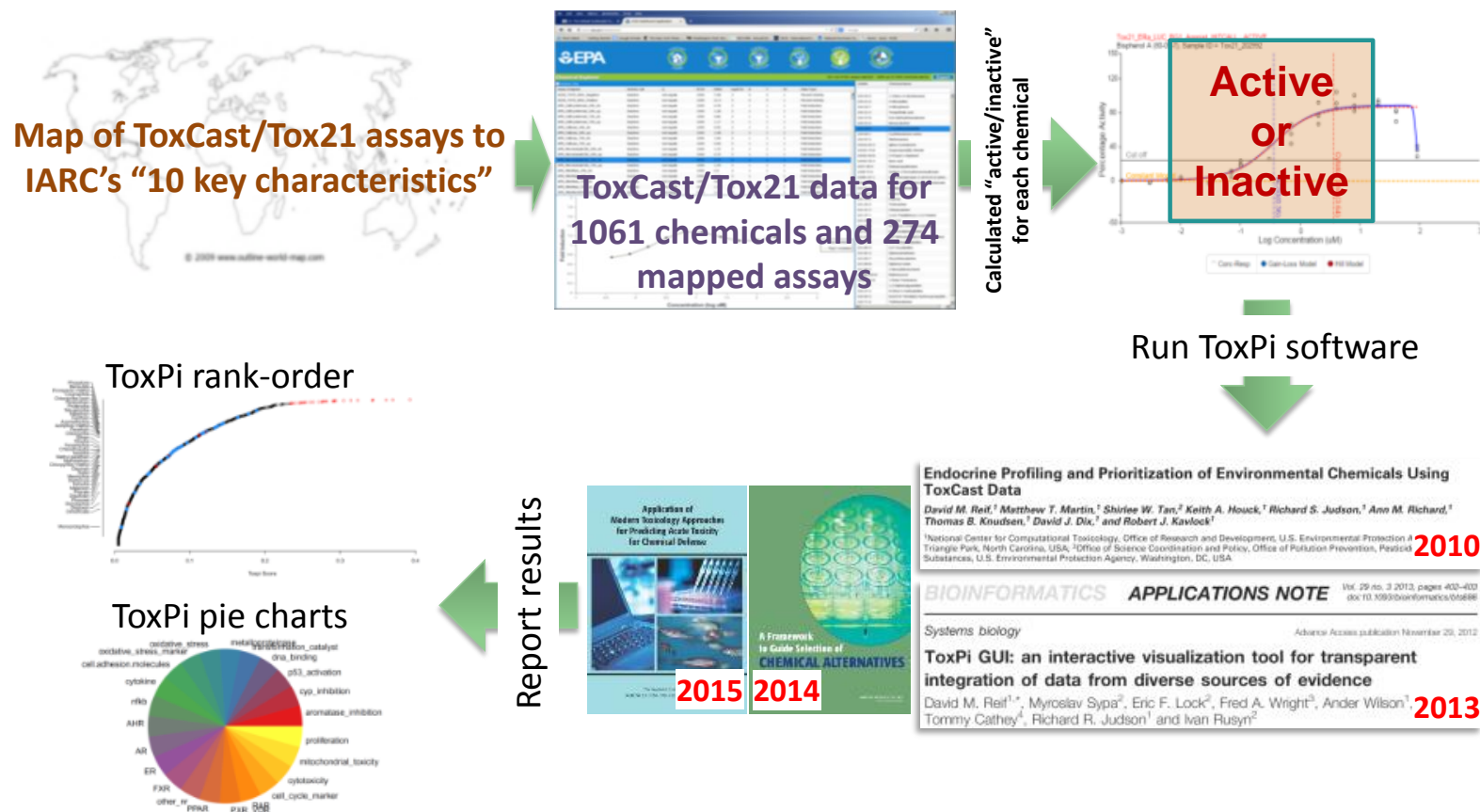
HTS assays do not support the hypothesis that PFOA predominantly activates the PPAR family of receptors, instead providing additional support for the hypothesis that multiple molecular pathways are operative.

Case Studies 2 & 3 Workflow

Mechanistic Questions:

- Are HTS data consistent with and/or supportive of the other available data on the 10 key mechanistic characteristics of carcinogens?
- How does activity compare to other compounds evaluated by IARC in this and previous monographs?
- Is activity more closely associated with the parent compound or a metabolite?

Approach/Workflow:

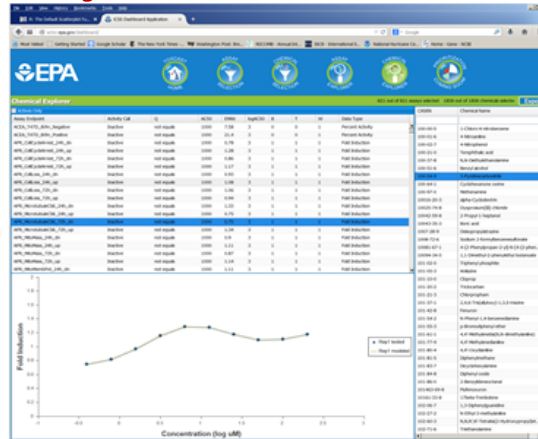


Mapping Key Characteristics to Assays

ToxCast iCSS dashboard

(<http://actor.epa.gov/dashboard/>)

- 821 assays
- 1860 chemicals
- Data are fully exportable



- 3 experts mapped each assay to 10 “key characteristics”
- 3 additional experts reviewed mapping and made suggestions
- Consensus cross-reference of assays to “key characteristics” and sub-categories was developed

At most, 274 ToxCast/Tox21 assays could be mapped to a “key characteristic”:

Key characteristic	1. Is electrophilic or can be metabolically activated	2. Is genotoxic (considered for V112, but not for V113)	4. Induces epigenetic alterations	5. Induces oxidative stress	6. Induces chronic inflammation	8. Modulates receptor-mediated effects	10. Alters cell proliferation, cell death and nutrient supply
Assay Endpoints	31 assays: • CYP inhibition (29) • Aromatase inhib. (2)	[9 assays: • p53 activation]	11 assays: • DNA binding (4) • Transformation (7)	18 assays: • Metalloproteinase (5) • Oxidative stress (7) • Oxidative stress marker (6)	45 assays: • Cell adhesion (14) • Cytokines (29) • NFkB (2)	81 assays: • AhR (2) • AR (11) • ER (18) • FXR (7) • Others (18) • PPAR (12) • PXR_VDR (7) • RAR (6)	68 assays: • Cell cycle (16) • Cytotoxicity (41) • Mitochondrial toxicity (7) • Proliferation (4)

No assay coverage for these “key characteristics”

3. Alters DNA repair or causes genomic instability

7. Immunosuppressant

9. Immortalization

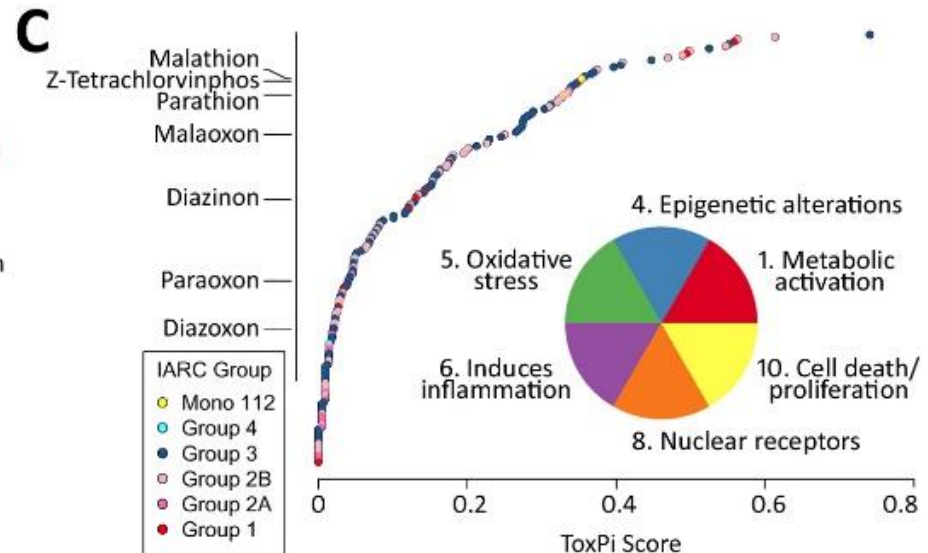
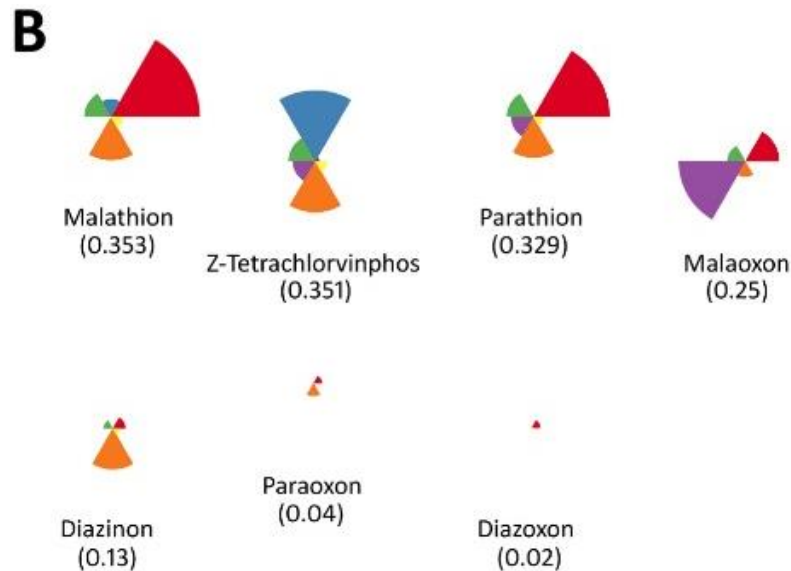
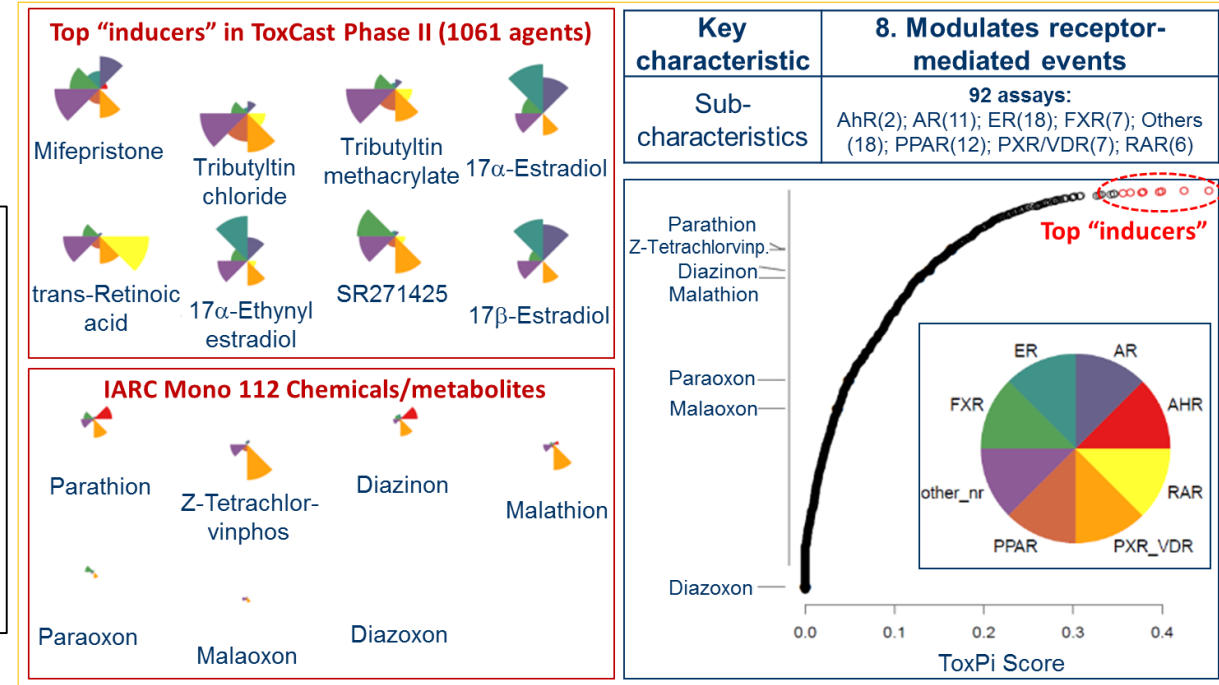
Limited coverage for two additional “key characteristics”

Example Results for Vol 112

Volume 112 (Diazinon):

Diazinon demonstrated activity in both AhR assays and additional effects in a subset of estrogen receptor alpha and beta assay endpoints.

Diazoxon exhibited little activity across the 274 assay endpoints, but high reactivity and short half-life make interpretations difficult.

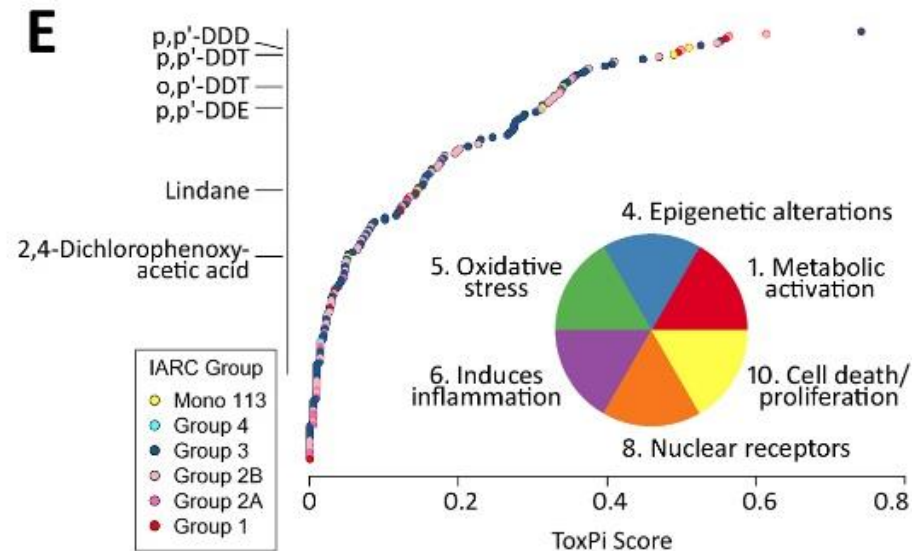
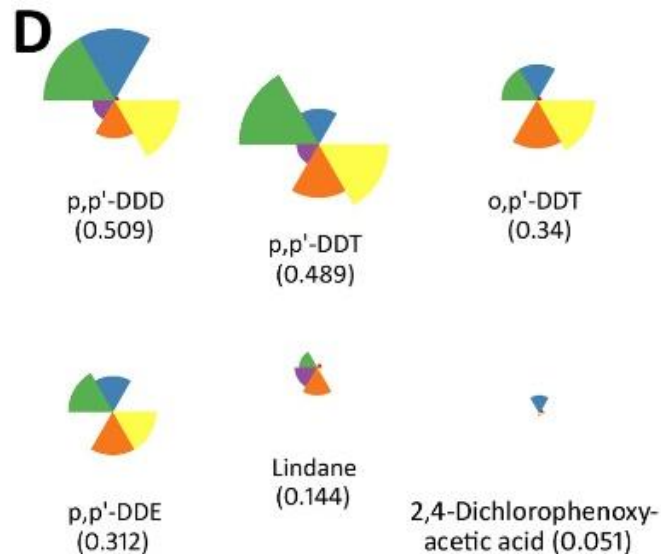
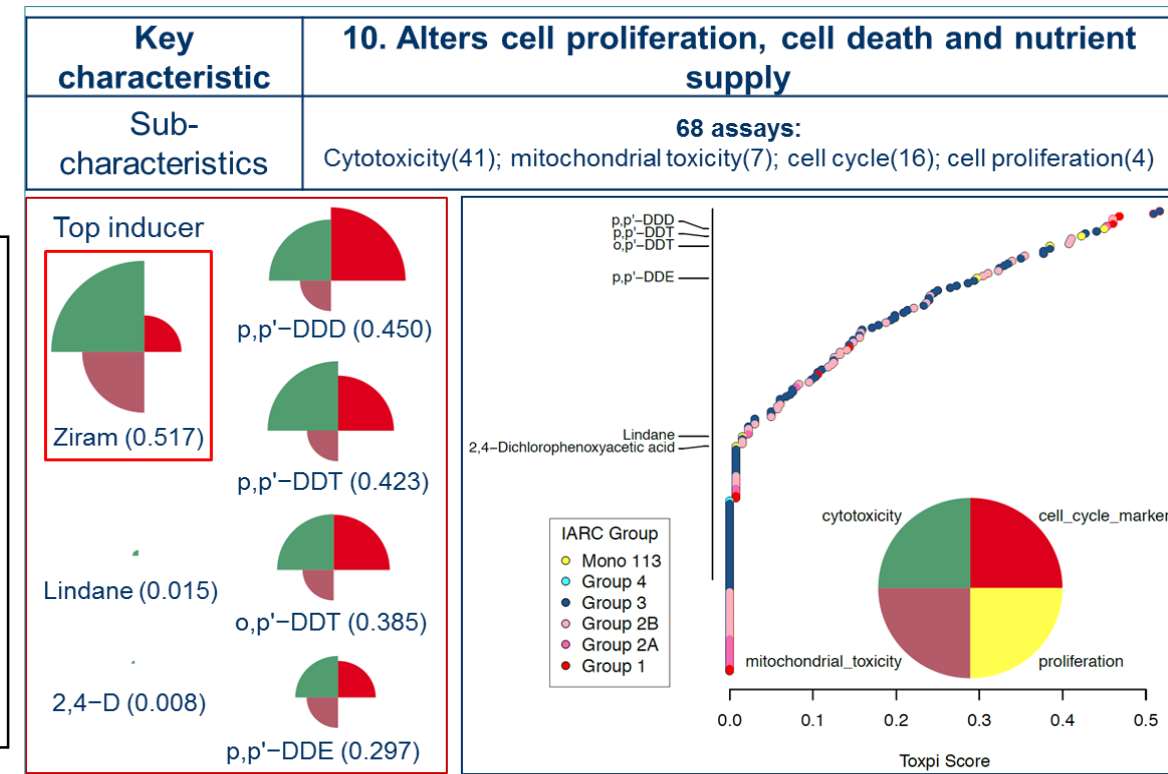


Example Results for Vol 113

Volume 113 (DDT):

p,p'-DDT, **o,p'-DDT**, **p,p'-DDE**, and **p,p'-DDD** were positive in between 42 and 62 high throughput assays, mostly related to receptor-mediated effects or cell proliferation/cell death/nutrient supply.

All four compounds had similar activity profiles (both in terms of overall rank and “shape” of ToxPi) (note contrast with diazinon and diozoxon).



Conclusions

- These case studies demonstrate several applications of **high throughput *in vitro* toxicity data** in the evaluation of carcinogenic mechanisms.
- Screening data are particularly well suited for making comparisons across chemicals and across endpoints due to their **wide coverage**.
- Evaluations of high throughput data are enhanced by organizing around the **“key characteristics of carcinogens”** (Smith et al., 2016).
- Needs for the future
 - **Gaps in assay coverage** in the context of evaluating carcinogenicity clearly need to be addressed.
 - **Data analysis advances** such as incorporation of formal multivariate clustering, potency estimates, and dosimetry considerations can further improve the informativeness of high throughput *in vitro* toxicity data for mechanistic evaluations.

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