USE OF HIGH THROUGHPUT SCREENING DATA IN INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC) MONOGRAPH EVALUATIONS

Ivan Rusyn[1], Weihsueh Chiu[1], Kate Guyton[2], Mathew Martin[3], David Reif[4]

[1]Texas A & M University, College Station, TX, [2]International Agency for Research on Cancer, Lyon, France, [3]US Environmental Protection Agency, Durham, NC, [4]North Carolina State University, Raleigh, NC

DATA INTEGRATION IN IARC EVALUATIONS OF HUMAN CANCER HAZARD

Step 1: Sub-group review and evaluation

Cancer in humans	Cancer in experimental animals	Mechanistic and other relevant data
• *Sufficient evidence* • *Limited evidence* • *Inadequate evidence* • *Evidence suggesting lack of carcinogenicity*	• *Sufficient evidence* • *Limited evidence* • *Inadequate evidence* • *Evidence suggesting lack of carcinogenicity*	• Data for each key characteristic are "weak," "moderate," or "strong"? • Determine whether the identified mechanisms could operate in humans

Step 2: Working Group review and evaluation during Plenary session

Overall evaluation
[Sub-group evaluations are discussed, revised and adopted]

- 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*
- Group 4 *Probably not carcinogenic to humans*

EVIDENCE IN EXPERIMENTAL ANIMALS

EVIDENCE IN HUMANS	*Sufficient*	*Limited*	*Inadequate*	*ESLC*
Sufficient	Group 1			
Limited	↑1 strong evidence in exposed humans **Group 2A**	↑2A belongs to a mechanistic class where other members are classified in Groups 1 or 2A **Group 2B** (exceptionally, Group 2A)		
Inadequate	↑1 strong evidence in exposed humans ↑2A strong evidence … mechanism also operates in humans **Group 2B** ↓3 strong evidence … mechanism does not operate in humans	↑2A belongs to a mechanistic class ↑2B with supporting evidence from mechanistic and other relevant data **Group 3**	↑2A belongs to a mechanistic class ↑2B with strong evidence from mechanistic and other relevant data **Group 3**	**Group 3** ↓4 consistently and strongly supported by a broad range of mechanistic and other relevant data
ESLC	Group 3			**Group 4**

All pertinent **epidemiological** studies and **cancer bioassays in rodents**

- Study designs and results are detailed in tables
- Descriptions of individual studies are in text [*comments in brackets*]

Representative **mechanistic data** judged to be important by the Working Group

- Includes information on (i) toxicokinetics, (ii) **representative data on the 10 key characteristics of carcinogens**, (iii) **data relevant to comparisons across agents and end-points**, (iv) cancer susceptibility, and (v) **other adverse effects**
- Mechanistic and other relevant data for the agent under consideration are drawn from representative studies in humans, animals, and ***in vitro***
- Written in the form of a review article [*comments in brackets*]

All studies must be *publicly available* (published or accepted)

- Includes studies published in languages other than English
- Does not consider research in progress, articles in preparation, consultant reports, or anything that is not publicly available
- **Databases (e.g. PubChem) and peer-reviewed government reports can also be searched**

IARC EVALUATIONS: QUESTIONS ADDRESSED WITH MECHANISTIC DATA

Main questions that need to be addressed:

- Is there strong evidence of an operative carcinogenic mechanism(s)?
- Is the evidence from exposed humans, human *in vitro* systems, or animals?
- Does the mechanism only operate in animals?
- Does the agent belong to a class of agents evaluated as Group 1 or Group 2A?

Additional questions that often come up:

- Are there data gaps in mechanistic information?
- There appears to be an imbalance in the number of studies on different mechanisms; is it because other mechanisms received less attention/funding, or because they are not operational/relevant?

http://monographs.iarc.fr/ENG/Preamble/instructions.php
http://www.toxicology.org/events/shm/fda/docs/FDA4_VJCogliano.pdf

IARC MONOGRAPH VOLUME 100E

Acetaldehyde: Possibly carcinogenic to humans (Group 2B) Vol. 71

Acetaldehyde associated with the consumption of alcoholic beverages: Carcinogenic to humans (Group 1) Vol. 100E

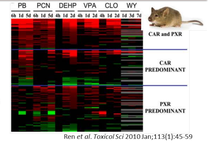
The Working Group (Vol. 100E) considered the following:

- *"Upon ingestion of alcoholic beverages, ethanol is converted into acetaldehyde, which is then oxidized to acetate*
- *Ethanol and acetaldehyde are both carcinogenic in experimental animals*
- *There is sufficient epidemiological evidence showing that humans who are deficient in the oxidation of acetaldehyde to acetate have a substantially increased risk for development of alcohol-related cancers, in particular of the oesophagus and the upper aerodigestive tract"*

IARC MONOGRAPH VOLUME 101

Di-ethylhexyl phthalate (DEHP):

"…the Working Group concluded that the human relevance of the molecular events leading to DEHP induced cancer in several target tissues (e.g., liver and testis) in rats or mice could not be ruled out, resulting in the evaluation of DEHP as a ***Group 2B agent, rather than Group 3****"*



Ren *et al. Toxicol Sci* 2010 Jan;113(1):45-59

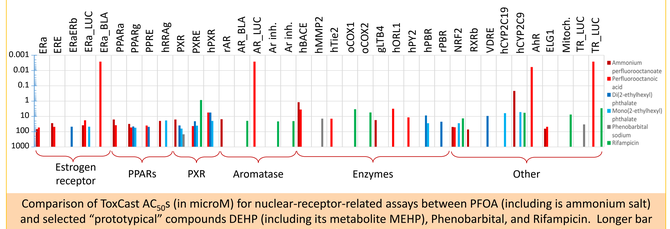
USING *IN VITRO* SCREENING DATA IN THE IARC VOLUME 110 – PFOA

Mechanistic Question:

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

How to answer:

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



Comparison of ToxCast AC_{50}s (in microM) for nuclear-receptor-related assays between PFOA (including is ammonium salt) and selected "prototypical" compounds DEHP (including its metabolite MEHP), Phenobarbital, and Rifampicin. Longer bar indicates greater potency (lower AC_{50}). Assays for which all compounds were negative are not displayed.

KEY CHARACTERISTICS OF KNOWN HUMAN CARCINOGENS AND THEIR RELATIONSHIP TO TOXCAST/TOX21

Key characteristic	Example of relevant evidence
1. Electrophilic or ability to undergo metabolic activation	Parent compound or metabolite with an electrophilic structure (epoxide, quinone, etc.), formation of DNA and protein adducts
2. Genotoxic	DNA damage, intercalation, gene mutations, cytogenetic changes (chromosome aberrations, micronucleus formation)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g. topoisomerase II, base-excision or double-strand break repair)
4. Epigenetic Alterations	DNA methylation, histone modification, microRNAs
5. Oxidative Stressor	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g. DNA, proteins, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Immunosuppressant	Decreased immuno-surveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g. ER, PPAR, AhR) or modulation of exogenous ligands (including hormones)
9. Immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death, or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell-cycle control, angiogenesis

Smith et al., ***Environ Health Perspect*** (in press) and http://monographs.iarc.fr/ENG/Preamble/previous/Instructions_to_Authors_54.pdf

ToxCast iCSS dashboard
(http://actor.epa.gov/dashboard/)

- 821 assays
- 1860 chemicals
- Data are fully exportable

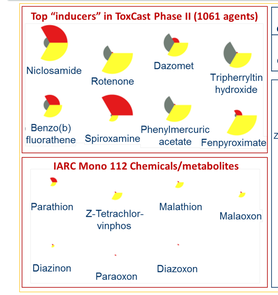
- 3 experts mapped each assay to 10 "key characteristics"
- 3 additional experts reviewed mapping and made suggestions
- Consensus cross-reference of assays to "key characteristics" and sub-categories was developed

274 ToxCast/Tox21 assays mapped to "key characteristics" of known human carcinogens:

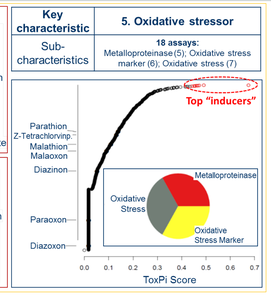
Key characteristic	1. Electrophilic or ability to undergo metabolic activation	2. Genotoxic* (*, considered in v.112, but not in v.113)	4. Causes Epigenetic alterations	5. Oxidative stressor	6. Induces chronic inflammation	8. Modulates receptor-mediated effects	10. Alters cell proliferation, cell death and nutrient supply
Sub-characteristics	**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)	**92 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)

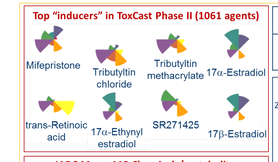
No coverage for these "key characteristics" → **3. Alters DNA repair or causes genomic instability** | **7. Immunosuppressant** | **9. Immortalization**

TOXCAST/TOX21 DATA IN THE IARC VOLUME 112

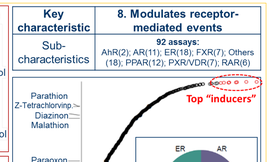


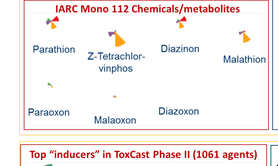
Key characteristic	5. Oxidative stressor
Sub-characteristics	**18 assays:** Metalloproteinase (5); Oxidative stress marker (6); Oxidative stress (7)



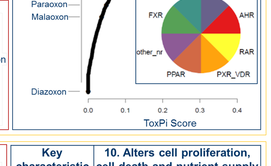


Key characteristic	8. Modulates receptor-mediated events
Sub-characteristics	**92 assays:** AhR(2); AR(11); ER(18); FXR(7); Others (18); PPAR(12); PXR/VDR(7); RAR(6)





Key characteristic	10. Alters cell proliferation, cell death and nutrient supply
Sub-characteristics	**68 assays:** Cell cycle (16); Cytotoxicity (41); Mitochondrial toxicity(7); Proliferation(4)



IARC Monograph Volume 112 (Diazinon):
*"Overall, **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 with only 3 assay endpoints found as active. The limited activity of diazoxon may be attributed to high reactivity and short half-life of this compound making interpretation of the results of the assay endpoints difficult."*

IARC Monograph 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, among the 265 assay endpoints relevant to the key characteristics of human carcinogens."*

CONCLUSIONS

- These case studies provide a demonstration of several potential applications of *in vitro* toxicity data in the evaluation of carcinogenic mechanisms. Because such screening data are available across a wide range of chemicals, they are particularly well suited for making comparisons across chemicals and across endpoints.
- Examining *in vitro* toxicity data in the context of the recently developed "key characteristics of carcinogens" (Smith et al., 2015) provides an even more powerful approach for mechanistic evaluations.
- "Mapping" of *in vitro* assays to "key characteristics of carcinogens" clearly delineates some of the gaps in assay availability in the context of evaluating carcinogenicity.
- There are number of methodological approaches that could help to further improve the informativeness of the *in vitro* toxicity data for mechanistic evaluations. For instance, more formal multivariate clustering or similarity analyses could be developed that would provide more information than the ToxPi score and rank alone in making comparisons across compounds.

ACKNOWLEDGEMENTS

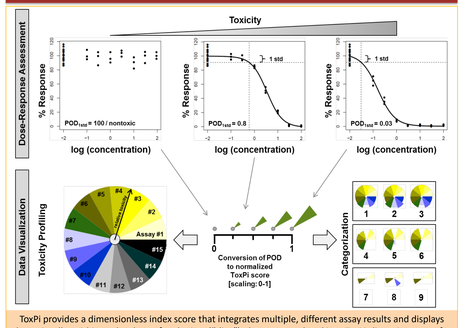
The authors acknowledge invaluable contributions from the Working Group members for IARC Monographs 110, 112 and 113, IARC Monographs Programme staff, and Dr. Keith Houck of US EPA.

REFERENCES

- IARC Preamble and full text monographs: http://monographs.iarc.fr/
- 10 Key Characteristics of Human Carcinogens: Smith et al @ PMID: 26600562
- ToxPi approach and software: Reif et al @ PMID: 20826373 and PMID: 23202747
- ToxCast data: http://epa.gov/ncct/toxcast/data.html
- EPA iCSS Dashboard for data access: http://actor.epa.gov/dashboard/
- SOT-FDA Colloquia: http://toxicology.org/events/shm/fda/fdacolloquia.asp

QR code

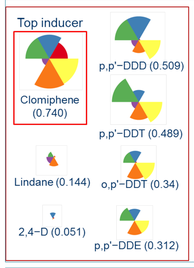
DATA INTEGRATION WITH TOXPI

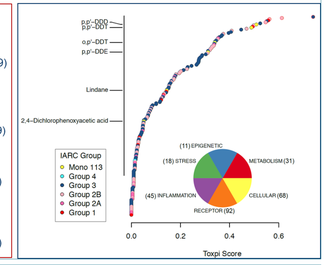


ToxPi provides a dimensionless index score that integrates multiple, different assay results and displays them visually. Within each subset of endpoints ("slice"), data are translated into ToxPi slice-wise scores for all compounds. Within each individual slice for a given chemical, the distance from the origin represents the relative chemical-elicited activity of the component assays. The overall score for a chemical, visualized as a radial ToxPi profile, is the aggregation of all slice-wise scores. (Reif et al., 2013)

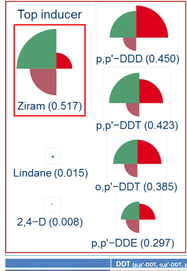
TOXCAST/TOX21 DATA IN THE IARC VOLUME 113

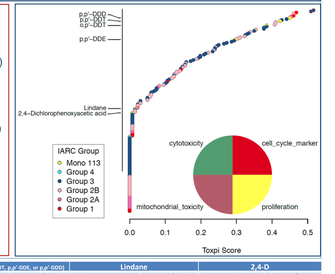
Key characteristics	**265 ToxCast/Tox21 assays:** Metabolism (31); Cell prolifration or death (68); Receptor-mediated events (92); Inflammation (45); Oxidative Stress (18); Epigenetics (11)





Key characteristic	10. Alters cell proliferation, cell death and nutrient supply
Sub-characteristics	**68 assays:** Cytotoxicity(41); mitochondrial toxicity(7); cell cycle(16); cell proliferation(4)





	DDT (p,p'-DDT; o,p'-DDT; p,p'-DDD)	Lindane	2,4-D
Key Characteristic	**Overall evidence**	**ToxCast/Tox21**	**Overall evidence**	**ToxCast/Tox21**	**Overall evidence**	**ToxCast/Tox21**
1. Is Electrophilic or Can Be Metabolically Activated	Inadequate data	0 or 1 out of 29 assays	Inadequate data	1 out of 29 assays	Inadequate data	1 out of 29 assays
2. Is Genotoxic	Moderate		Moderate		Weak	
3. Alters DNA repair or causes genomic instability	Inadequate data		Inadequate data		Inadequate data	
4. Induces Epigenetic Alterations	Inadequate data	2 or 4 out of 11 assays	Inadequate data	0 out of 11 assays	Inadequate data	1 out of 11 assays
5. Induces Oxidative Stress	Strong – can operate in humans	4 to 8 out of 18 assays	Strong, can operate in humans	2 out of 18 assays	Strong, can operate in humans	0 out of 18 assays
6. Induces chronic inflammation	Moderate	0 or 1 out of 45 assays	Weak	1 out of 45 assays	Inadequate data	0 out of 45 assays
7. Is Immunosuppressive	Strong – can operate in humans		Strong – can operate in humans		Moderate	
8. Modulates receptor-mediated effects	Strong – can operate in humans	15 to 21 out of 92 assays	Moderate	11 out of 92 assays	Weak	1 out of 92 assays
9. Causes Immortalization	Inadequate data		Inadequate data		Inadequate data	
10. Alters cell proliferation, cell death or nutrient supply	Moderate	17 to 27 out of 68 assays	Weak	2 out of 68 assays	Weak	1 out of 68 assays