Use of Computational and *In Vitro* Data in Cancer Hazard Assessment of Data-Rich Chemicals: Examples of IARC Monographs

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Conflict of Interest Statement

1. The author declares no relevant conflicts of interest with respect to the content of this presentation.

2. This presentation does not reflect the official views of WHO, IARC, Texas A&M University, or any other organization or a third party, and contains personal opinions of Dr. Rusyn.

3. The information in this presentation, as it pertains to evaluations by IARC Monographs Programme, is not final and may be subject to changes pending final editing of the Monographs.
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Data are from Tox21 and ToxCast toxicity testing programs
IARC Evaluations: Sources of Data

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 Monographs for Evaluation of Cancer Hazard in Humans

Preamble

General Remarks

Several *Monographs* in one volume:

1. Exposure data
2. Cancer in humans
3. Cancer in animals
4. Mechanistic and other relevant data
5. Summary
6. Evaluation and rationale

References

http://www.toxicology.org/events/shm/fda/docs/FDA4_KZGUYTON.pdf
Data Integration in IARC Evaluations

**Step 1: Sub-group review and evaluation**

- **Cancer in humans**
  - Sufficient evidence
  - Limited evidence
  - Inadequate evidence
  - Evidence suggesting lack of carcinogenicity

- **Cancer in experimental animals**
  - Sufficient evidence
  - Limited evidence
  - Inadequate evidence
  - Evidence suggesting lack of carcinogenicity

- **Mechanistic and other relevant data**
  - 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
## Data Integration in IARC Evaluations: Role of “Mechanistic Evidence”

<table>
<thead>
<tr>
<th>Evidence in Humans</th>
<th>Evidence in Experimental Animals</th>
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<tbody>
<tr>
<td>Sufficient</td>
<td><strong>Group 1</strong></td>
</tr>
<tr>
<td>Limited</td>
<td><strong>Group 2A</strong></td>
</tr>
<tr>
<td>Inadequate</td>
<td><strong>Group 2B</strong></td>
</tr>
<tr>
<td><strong>ESLC</strong></td>
<td><strong>Group 4</strong></td>
</tr>
</tbody>
</table>

### Sufficient Evidence
- **Group 1**
  - 2A belongs to a mechanistic class where other members are classified in Groups 1 or 2A

### Limited Evidence
- **Group 2A**
  - 1 strong evidence in exposed humans

### Inadequate Evidence
- **Group 2B**
  - 1 strong evidence in exposed humans
  - 2A strong evidence ... mechanism also operates in humans

### Evidentc in Experimental Animals
- **Group 3**
  - 2A belongs to a mechanistic class
  - 2B with supporting evidence from mechanistic and other relevant data

- **Group 4**
  - 4 consistently and strongly supported by a broad range of mechanistic and other relevant data
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
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.

Each panel shows AC50s (microM concentrations) from in vitro assays reported on the EPA iCSS Dashboard (http://actor.epa.gov/dashboard/) for PFOA or its ammonium salt and several prototypical nuclear receptor activators: rifampicin (PXR), phenobarbital (CAR), DEHP and MEHP (PPARs). All assays in which positive results were obtained for at least one of the six compounds were included in the analysis.
Chapter 4 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.

Results were subdivided into six groups by each molecular target as follows:

- estrogen receptor,
- PPAR,
- PXR,
- aromatase,
- enzyme assays, and
- other receptors

Example conclusion based on ToxCast data in Monograph 110:

*Volume 110 (PFOA):* Liver toxicity observed in rodents has been associated with both PPARα-dependent and -independent mechanisms. *The Working Group’s analysis of human in vitro data is consistent with multiple molecular pathways being operative.*
# IARC Evaluations: Key Characteristics of Known Human Carcinogens

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

Smith et al., *Environ Health Perspect* (in press) and [http://monographs.iarc.fr/ENG/Preamble/previous/Instructions_to_Authors_S4.pdf](http://monographs.iarc.fr/ENG/Preamble/previous/Instructions_to_Authors_S4.pdf)
IARC Evaluations: Identifying Data Gaps

Systematic literature search tree for the Glyphosate Monograph:
Last searches were conducted March 2, 2015

Key characteristic
1. Electrophilic or ability to undergo metabolic activation
2. Genotoxic
3. Alters DNA repair or causes genomic instability
4. Epigenetic Alterations
5. Oxidative Stressor
6. Induces chronic inflammation
7. Immunosuppressant
8. Modulates receptor-mediated effects
9. Immortalization
10. Alters cell proliferation, cell death, or nutrient supply

https://hawcproject.org/
Use of Tox21/ToxCast Data in IARC Monographs: Overall Workflow

Map of ToxCast/Tox21 assays to IARC’s “10 key characteristics”

ToxCast/Tox21 data for 1061 chemicals and 274 mapped assays

ToxPi rank-order

ToxPi pie charts

Calculated “active/inactive” for each chemical

Run ToxPi software

Active or Inactive

Report results
Use of Tox21/ToxCast Data in IARC Monographs: Mapping ToxCast/Tox21 Assays to “Characteristics”

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:

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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31 assays: • CYP inhibition (29) • Aromatase inhib. (2)</td>
<td>9 assays: • p53 activation</td>
<td>11 assays: • DNA binding (4) • Transformation (7)</td>
<td>-18 assays: • Metalloproteinase (5) • Oxidative stress (7) • Oxidative stress marker (6)</td>
<td>45 assays: • Cell adhesion (14) • Cytokines (29) • NFkB (2)</td>
<td>92 assays: • AhR (2) • Others (18) • AR (11) • PPAR (12) • ER (18) • PXR_VDR (7) • FXR (7) • RAR (6)</td>
<td>68 assays: • Cell cycle (16) • Cytotoxicity (41) • Mitochondrial toxicity (7) • Proliferation (4)</td>
</tr>
</tbody>
</table>

No assay coverage for these “key characteristics”

3. Alters DNA repair or causes genomic instability
7. Immunosuppressant
9. Immortalization

SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety
Visualizing Complex Information: ToxPi

Each chemical signature gives a Toxicological Priority Index (ToxPi) score that is useful for ranking chemicals.

ToxPi = \sum_{i=1}^{I} w_i \cdot assay_i + \sum_{c=1}^{C} w_c \cdot chemProp_c + \sum_{p=1}^{P} w_p \cdot pathway_p

ToxPi = f(In vitro assays + Chemical properties + Pathways)

[Reif et al. (2010) EHP]
Dose-Response Assessment

Toxicity Profiling

Visualizing Complex Information: ToxPi

Conversion of POD to normalized ToxPi score [scaling: 0-1]

SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety
Visualizing Complex Information: ToxPi

309 Chemicals sorted by ToxPi

Linuron

Pyrimethanil

Tebuthiuron

Methoxychlor

Rotenone

Bisphenol A

HPTE

[Reif et al. (2010) EHP]
ToxPi Analysis Based on 10 “Characteristics”: Similarity Among Agents

Prochloraz

Imazalil

Clotrimazole
ToxPi Analysis Based on 10 “Characteristics”: Common Pathways

- Oxidative stress & cytotoxicity
  - Tributyltin methacrylate
  - Tributyltin chloride
  - Niclosamide

- Estrogen receptor activation
  - 17α-Ethinylestradiol
  - Diethylstilbestrol
  - Bisphenol AF
Use of Tox21/ToxCast Data in IARC Monographs: General Considerations from Volumes 112 and 113

- Most agents (and their key metabolites and/or isomers) that were evaluated in IARC Monographs 112 (March 2015) and 113 (June 2015) were 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)

- The match of an assay to the “key characteristic” was to provide additional insights into the bioactivity profile of a chemical under evaluation with respect to its potential to interact with, or have an effect on, targets that may be associated with carcinogenesis in humans

- For each chemical the results of the in vitro assays that represent each “key characteristic” can be compared to the results for a larger compendium of substances with similar in vitro data (either those screened in ToxCast/Tox21 or those that have been previously evaluated in the IARC monographs and were screened in ToxCast)
Use of Tox21/ToxCast Data in IARC Monographs: Examples from Volume 112

Top “inducers” in ToxCast Phase II (1061 agents)
- Niclosamide
- Rotenone
- Dazomet
- Tripherryltin hydroxide
- Benzo(b) fluoranthene
- Spiroxamine
- Phenylmercuric acetate
- Fenpyroximate

IARC Mono 112 Chemicals/metabolites
- Parathion
- Z-Tetrachlorvinphos
- Malathion
- Malaoxon
- Diazinon
- Paraoxon
- Diazoxon

Key characteristic

<table>
<thead>
<tr>
<th>Sub-characteristics</th>
<th>5. Oxidative stressor</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 assays: Metalloproteinase (5); Oxidative stress marker (6); Oxidative stress (7)</td>
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</table>
Use of Tox21/ToxCast Data in IARC Monographs: Examples from Volume 112

<table>
<thead>
<tr>
<th>Top “inducers” in ToxCast Phase II (1061 agents)</th>
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<tbody>
<tr>
<td>Mifepristone</td>
</tr>
<tr>
<td>Tributyltin chloride</td>
</tr>
<tr>
<td>trans-Retinoic acid</td>
</tr>
<tr>
<td>17α-Ethynyl estradiol</td>
</tr>
<tr>
<td>17α-Estradiol</td>
</tr>
<tr>
<td>SR271425</td>
</tr>
<tr>
<td>17β-Estradiol</td>
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<th>8. Modulates receptor-mediated events</th>
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IARC Mono 112 Chemicals/metabolites

- Parathion
- Z-Tetrachlorvinphos
- Diazinon
- Malathion
- Paraoxon
- Malaoxon
- Diazoxon
- Malaoxon
- Paraoxon
- Malaoxon
Use of Tox21/ToxCast Data in IARC Monographs: Examples from Volume 112

**Top “inducers” in ToxCast Phase II (1061 agents)**

- Tributyltin chloride
- Niclosamide
- Tributyltin methacrylate
- Triphenyltin hydroxide
- Phenylmercuric acetate
- Tebufenpyrad
- Emamectin benzoate

**IARC Mono 112 Chemicals/metabolites**

- Z-Tetrachlorvinphos
- Malathion
- Parathion
- Diazinon
- Diazoxon
- Paraaxon
- Malaoxon

**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-evaluated compounds that have ToxCast/Tox21 data (n=178) | Key characteristic | 8. Modulates receptor-mediated events |
<table>
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<td></td>
<td>Sub-characteristics</td>
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</table>

**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.
Use of Tox21/ToxCast Data in IARC Monographs: Examples from Volume 113

<table>
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<th>Key characteristic</th>
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<td>Sub-characteristics</td>
<td>68 assays: Cytotoxicity (41); mitochondrial toxicity (7); cell cycle (16); cell proliferation (4)</td>
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DDT-related compounds share similar ToxPi **shape** and **overall rank**

Noticeably greater activity than lindane or 2,4-D
Use of Tox21/ToxCast Data in IARC Monographs: Examples from Volume 113

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<tr>
<th>Compound</th>
<th>Evidence from “traditional” data</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT</td>
<td><strong>Strong</strong>, can operate in humans</td>
</tr>
<tr>
<td>Lindane</td>
<td><strong>Moderate</strong></td>
</tr>
<tr>
<td>2,4-D</td>
<td><strong>Weak</strong></td>
</tr>
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**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.
Options for Accessing ToxCast/Tox21 data

http://www.epa.gov/ncct/toxcast/data.html

http://actor.epa.gov/edsp21/

Additional Information

ToxCast / Tox21: Characterizing chemical hazard using HTS

ToxCast data: http://epa.gov/ncct/toxcast/data.html

Source data used for IARC analysis:
http://epa.gov/ncct/toxcast/files/ToxCast%20Summary%20Files/ToxCast_Summary_Files.zip

Detailed information on source data:

ToxCast data processing overview:

Toxicological Prioritization Index (ToxPi): Visual analytic framework for data integration

ToxPi software and user manual available from: http://comptox.us/toxpi.php

Examples of models/rankings that have developed using ToxPi with/without ToxCast data:

Endocrine (using ToxCast Phase I data)

Cardiotoxicity (using externally collected in vitro data)
Sirenko et al. (2013) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3900303/

Ranking chemicals based on exposure information