Systematic identification of the mechanistic evidence for cancer hazard assessment: Experience of the IARC Monographs programme

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I declare no financial interests related to the subject matter of my presentation.
Presentation Overview

- IARC Monograph- background
- Challenges and recommendations for mechanistic data
- Recent experience in search and organisation of mechanistic information
  - Published literature
  - Tox21 data
- Summary
Agents are recommended by international advisors based on:
- Evidence of human exposure
- Some evidence or suspicion of carcinogenicity

More than 980 agents have been evaluated
- 118 are \textit{carcinogenic to humans} (Group 1)
- 75 are \textit{probably carcinogenic to humans} (Group 2A)
- 287 are \textit{possibly carcinogenic to humans} (Group 2B)
- 503 are \textit{not classifiable as to its carcinogenicity to humans} (Group 3)
- 1 is classified as \textit{probably not carcinogenic to humans} (Group 4)

National and international health agencies use the \textit{Monographs}
- To identify carcinogens
- To prevent exposure to known or suspected carcinogens
How Are IARC Monograph Evaluations Conducted?

- Procedural guidelines for participant selection, conflict of interest, stakeholder involvement & meeting conduct

- Separate criteria for review of human, animal and mechanistic evidence

- Decision process for overall evaluations

http://monographs.iarc.fr/ENG/Preamble/index.php
Cancer Hazard Assessment
Based on Three Lines of Evidence

Cancer in humans
Cancer in animals
Mechanisms

Overall evaluation

“Systematic approach to cancer hazard evaluation”:
- Systematic gathering and review of all lines of evidence
- Uniform, hierarchic evaluation structure
The IARC Monographs Evaluations: A Two-Step Process

**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?

**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

International Agency for Research on Cancer

World Health Organization
Mechanistic Data Are Pivotal When Human Data Are Not Sufficient (Example 1)

Evidence in Experimental Animals

- Sufficient
- Limited
- Inadequate

Evidence in Humans

- Sufficient
- Limited
- Inadequate

Group 1 (carcinogenic to humans)
Group 2A (probably carcinogenic)
Group 2B (possibly carcinogenic) (exceptionally, Group 2A)
Group 3 (not classifiable)

Strong supporting evidence in exposed humans
Mechanistic Data Are Pivotal When Human Data Are Not Sufficient (Example 2)

**EVIDENCE IN EXPERIMENTAL ANIMALS**

- **Sufficient**
  - Group 1 (*carcinogenic to humans*)
  - Group 2A (*probably carcinogenic*)
- **Limited**
  - Group 2B (*possibly carcinogenic*)
  - Exceptionally, Group 2A
- **Inadequate**
  - Group 2B (*possibly carcinogenic*)
  - Group 3 (*not classifiable*)

Strong evidence; mechanism also operates in humans
Mechanistic Data Are Pivotal When Human Data Are Not Sufficient (Example 3)

EVIDENCE IN EXPERIMENTAL ANIMALS

<table>
<thead>
<tr>
<th>Sufficient</th>
<th>Limited</th>
<th>Inadequate</th>
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<tbody>
<tr>
<td><strong>Sufficient</strong></td>
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<td></td>
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<tr>
<td><strong>Limited</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inadequate</strong></td>
<td></td>
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</tr>
</tbody>
</table>

- **Group 1** (*carcinogenic to humans*)
- **Group 2A** (*probably carcinogenic*)
- **Group 2B** (*possibly carcinogenic*)
- **Group 2B** (*possibly carcinogenic*; exceptionally, Group 2A)
- **Group 3** (*not classifiable*)

Strong evidence: mechanism in animals DOES NOT operate in humans
Insights from Volume 100 and Advisory Groups

• The volume and complexity of mechanistic evidence is increasing
• Analysis of high-throughput/-content data (including from curated government databases) is encouraged
• Objective methods to identify, select and evaluate mechanistic evidence are needed
• Although not necessarily representing mechanisms themselves, the key characteristics of human carcinogens can be used to advance systematic evaluation of relevant mechanistic data
Mechanistic Studies: Looking Forward

Considerations:
1. Monographs cite hundreds-thousands of studies
2. Evolution in experience over time:
   • Mail box(es) of papers (1970s-1980s era)
   • Electronic reference list, PDFs, database (1990s)
   • Sorted list of references by subject (early 2000s)

Challenges:
1. How, when, where were searches done?
2. Which studies were included/excluded?
3. So many mechanisms, so little time:
   • How to search systematically for relevant mechanisms?
   • How to bring uniformity across assessments (strength- but also lack of availability- of data)?
   • How to analyze the voluminous mechanistic database efficiently?
Strategy

1. Identify studies through documented searches
2. Organise the inventory of studies/data
3. Increase clarity in evidence summary and evaluations:
   - How much evidence? (“no evidence” vs “weak/moderate/strong”)
   - For what effects (which key characteristics)
   - In what tests (humans, in vitro, etc)
Step 1: Identify Studies through Well-Documented Searches

Information Sources:

1. **Literature**
   - Targeted literature searches on each key characteristic to address specific hypotheses
   - “Hand searching” for additional literature
     - General literature searches on the agent
     - Authoritative reviews (e.g., past Monographs)
     - Public submissions to “call for data”
     - Working Group

2. **Publicly available data** (e.g., ToxCast, Tox21, ToxRefDB, etc)
Step 1: Identify Studies through Well-Documented Searches

- Search for literature on each key characteristic
  - Terms developed with IARC, librarian, expert input
  - Expected to evolve over time (experience and MeSH tagging)
  - Mix of MeSH and text terms (facilitates updating before meeting)
- Complemented by “hand searching”
- Document searches and results using HAWC online tool (HAWCproject.org)
Step 2: Develop an Organized Inventory of Studies/Data

Organizing Principles:
- Topic (key characteristics)
- Species
- Utility
- Document exclusions
- Develop outline
- Identify experts/resources

IARC Vol 112- Mono 4- Glyphosate (2015): Literature Tagtree

Key characteristics of carcinogens:
- 142
- Is Genotoxic
- 5 Induces Oxidative Stress

Organizing Principles:
- Topic (key characteristics)
- Species
- Utility
- Document exclusions
- Develop outline
- Identify experts/resources
Step 2: Develop an Organized Inventory of Studies/Data

Compendium of endpoints and assays associated with each Key Characteristic

- Developed by IARC and experts
- Expected to evolve over time
Step 2: Develop an Organized Inventory of Studies/Data

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

- 821 assays
- 1860 chemicals

10 Key characteristics of human carcinogens:

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

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

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<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Endpoints</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>81 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>
<tr>
<td>3. Alters DNA repair or causes genomic instability</td>
<td>7. Immunosuppressant</td>
<td>9. Immortalization</td>
<td></td>
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</tr>
</tbody>
</table>

No assay coverage for these “key characteristics”
### Step 3: Summarize Mechanistic Evidence by Key Characteristic

<table>
<thead>
<tr>
<th>Key characteristic</th>
<th>8. Modulates receptor-mediated events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-characteristics</td>
<td>92 assays: AhR(2); AR(11); ER(18); FXR(7); Others (18); PPAR(12); PXR/VDR(7); RAR(6)</td>
</tr>
</tbody>
</table>

#### Volume 112 (Diazinon):

**Diazinon** demonstrated activity in both assays for AhR, and in a subset of estrogen receptor alpha and beta assay endpoints. **Diazoxon** exhibited little activity (may be attributable to high reactivity and short half-life).
Step 3: Summarize Mechanistic Evidence by Key Characteristic

**Example:** Glyphosate summary

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strength of evidence for glyphosate</th>
<th>Does this– or can it– operative in humans?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is Electrophilic or Can Be Metabolically Activated</td>
<td>Not electrophilic</td>
<td></td>
</tr>
<tr>
<td>2. Is Genotoxic</td>
<td>Strong</td>
<td>Can operate in humans</td>
</tr>
<tr>
<td>3. Alters DNA Repair or Causes Genomic Instability</td>
<td>No data</td>
<td></td>
</tr>
<tr>
<td>4. Induces Epigenetic Alterations</td>
<td>No data</td>
<td></td>
</tr>
<tr>
<td>5. <strong>Induces Oxidative Stress</strong></td>
<td>Strong</td>
<td>Can operate in humans</td>
</tr>
<tr>
<td>6. Induces Chronic Inflammation</td>
<td>No data</td>
<td></td>
</tr>
<tr>
<td>7. Is Immunosuppressive</td>
<td>Weak</td>
<td></td>
</tr>
<tr>
<td>8. Modulates Receptor-mediated Effects</td>
<td>Weak</td>
<td></td>
</tr>
<tr>
<td>9. Causes Immortalization</td>
<td>No data</td>
<td></td>
</tr>
<tr>
<td>10. Alters Cell Proliferation, Cell Death or Nutrient supply</td>
<td>Weak</td>
<td></td>
</tr>
</tbody>
</table>

“.. **Strong evidence** that glyphosate can operate through two key characteristics of known human carcinogens, and that these can be operative in humans”

http://monographs.iarc.fr/ENG/Monographs/vol112/mono112-09.pdf
## Summary of Mechanistic Evidence in Recent IARC Monographs Evaluations

<table>
<thead>
<tr>
<th>Agent</th>
<th>Human evidence</th>
<th>Animal evidence</th>
<th>Mechanistic evidence</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>Limited (NHL, leukemia, lung)</td>
<td>Limited</td>
<td>Genotoxicity, oxidative stress</td>
<td>2A</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>Limited (NHL)</td>
<td>Sufficient</td>
<td>Genotoxicity, oxidative stress</td>
<td>2A</td>
</tr>
<tr>
<td>Malathion</td>
<td>Limited (NHL, prostate)</td>
<td>Sufficient</td>
<td>Genotoxicity, oxidative stress, inflammation, receptor-mediated effects, and cell proliferation or death</td>
<td>2A</td>
</tr>
<tr>
<td>Parathion</td>
<td>Inadequate</td>
<td>Sufficient</td>
<td></td>
<td>2B</td>
</tr>
<tr>
<td>TCVP</td>
<td>Inadequate</td>
<td>Sufficient</td>
<td></td>
<td>2B</td>
</tr>
<tr>
<td>Lindane</td>
<td>Sufficient (NHL)</td>
<td>Sufficient</td>
<td>Immunosuppression</td>
<td>1</td>
</tr>
<tr>
<td>DDT</td>
<td>Limited (NHL, liver, testis)</td>
<td>Sufficient</td>
<td>Immunosuppression, oxidative stress, receptor-mediated effects</td>
<td>2A</td>
</tr>
<tr>
<td>2,4-D</td>
<td>Inadequate</td>
<td>Limited</td>
<td>Oxidative stress</td>
<td>2B</td>
</tr>
</tbody>
</table>
IARC Monographs: Example Timeline

**IARC Secretariat:**
- Coordinate all aspects of the Monograph development

**Working Group members:**
- Write the critical reviews and develop evaluations

**Invited Specialists:**
- Have critical knowledge but also a conflicting interest
  - [do not draft text or participate in evaluations]

**Representatives of national and international health agencies:**
- [do not draft text or participate in evaluations]

**Observers:**
- Allowed to observe but not to influence outcomes
  - [do not draft text or participate in evaluations]

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**IARC Secretariat:**
- Identify studies through well-documented searches
- Organize inventory of studies/data
- Recruit Working Group, organize and conduct meeting per published procedures

**Working Group members:**
- Perform supplemental literature searches
- Evaluate studies against published criteria
- Add comments [in square brackets]
- Draft assigned sections
- Peer-review

**Monograph in-person meeting:**
- Evidence summary and evaluation
- Plenary review and overall evaluation

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**Meeting announced (March 2014):**
- Preliminary List of Agents
- Call for Data and Experts
- Request for Observer Status
- WHO CoI form posted

**List of Participants announced (Jan. 2015)**

**The Lancet Oncology publication (March 2015)**

**References shared with health agencies (April 2015)**

**Glyphosate Monograph publication (July 2015)**
Summary: IARC Monographs

- Scientific findings providing insights into cancer mechanisms play an essential role in carcinogen hazard identification.

- The key characteristics of known human carcinogens provide the basis for an objective, systematic approach for identifying and evaluating mechanistic data.

- Recent IARC Monographs evaluations have illustrated the applicability of this approach.

- These developments lay groundwork for future evaluations where such data may fill important gaps in evidence of carcinogenicity.
Acknowledgments

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Thank YOU– and happy holidays!