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

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Conflict of Interest Statement

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

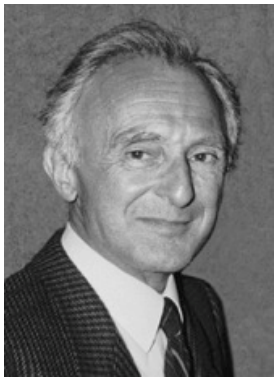
IARC Monographs –Presentation Outline

- IARC Monograph- background
- Challenges and recommendations for mechanistic data
- Recent experience in search and organisation of mechanistic information
  - Published literature
  - Tox21 data
- Summary

“The Encyclopaedia of Carcinogens”

Agents are recommended by international advisors based on:

- Evidence of human exposure
- Some evidence or suspicion of carcinogenicity



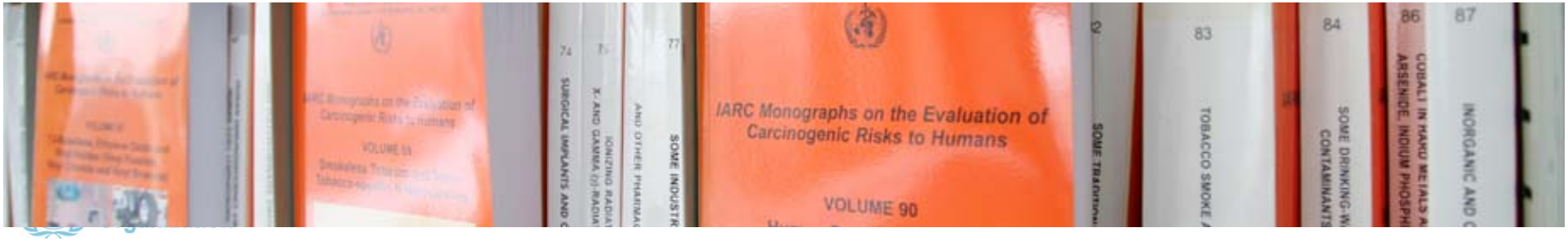
Lorenzo Tomatis
1929-2007

More than 980 agents have been evaluated

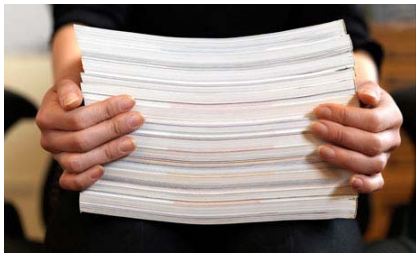
- 118 are ***carcinogenic to humans*** (Group 1)
- 75 are ***probably carcinogenic to humans*** (Group 2A)
- 287 are ***possibly carcinogenic to humans*** (Group 2B)
- 503 are ***not classifiable as to its carcinogenicity to humans*** (Group 3)
- 1 is classified as ***probably not carcinogenic to humans*** (Group 4)

National and international health agencies use the *Monographs*

- To identify carcinogens
- To prevent exposure to known or suspected carcinogens



How are the IARC Monograph Evaluations Conducted?



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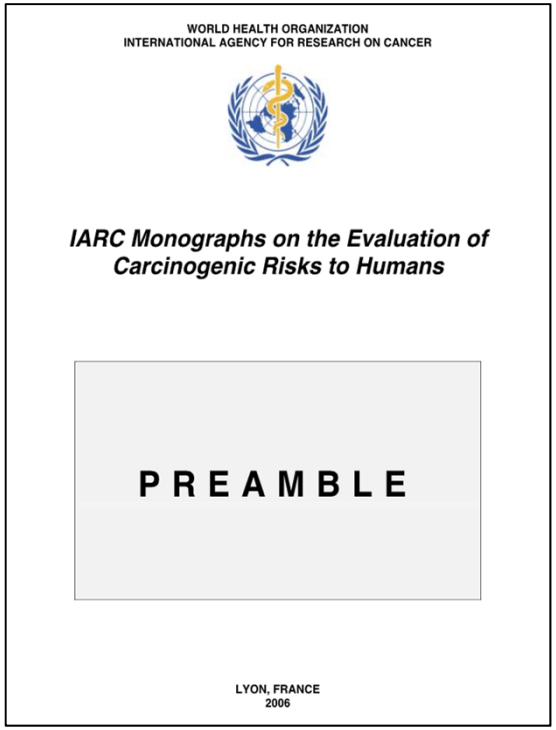


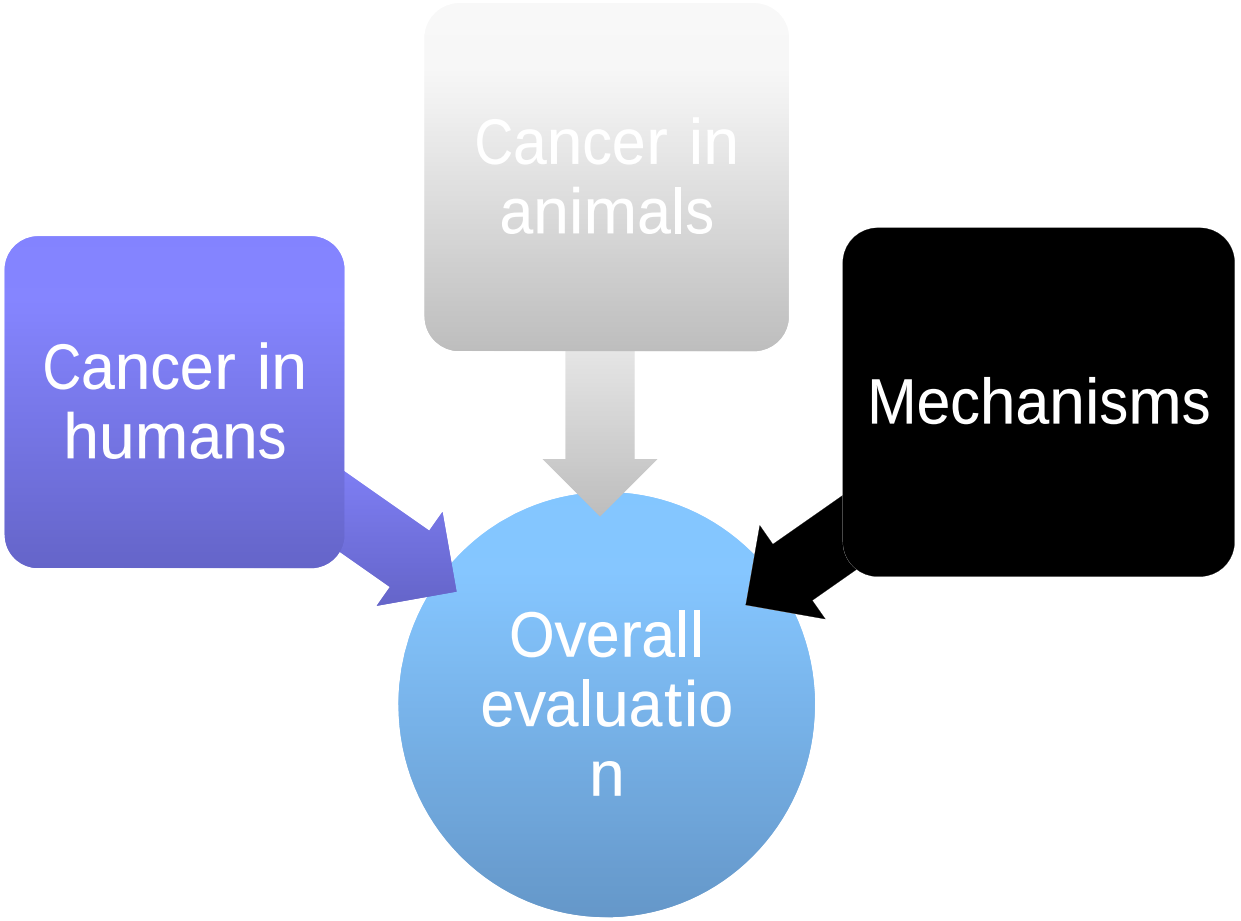
Internatio ch on Cancer

World Health Organization

- 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



"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*

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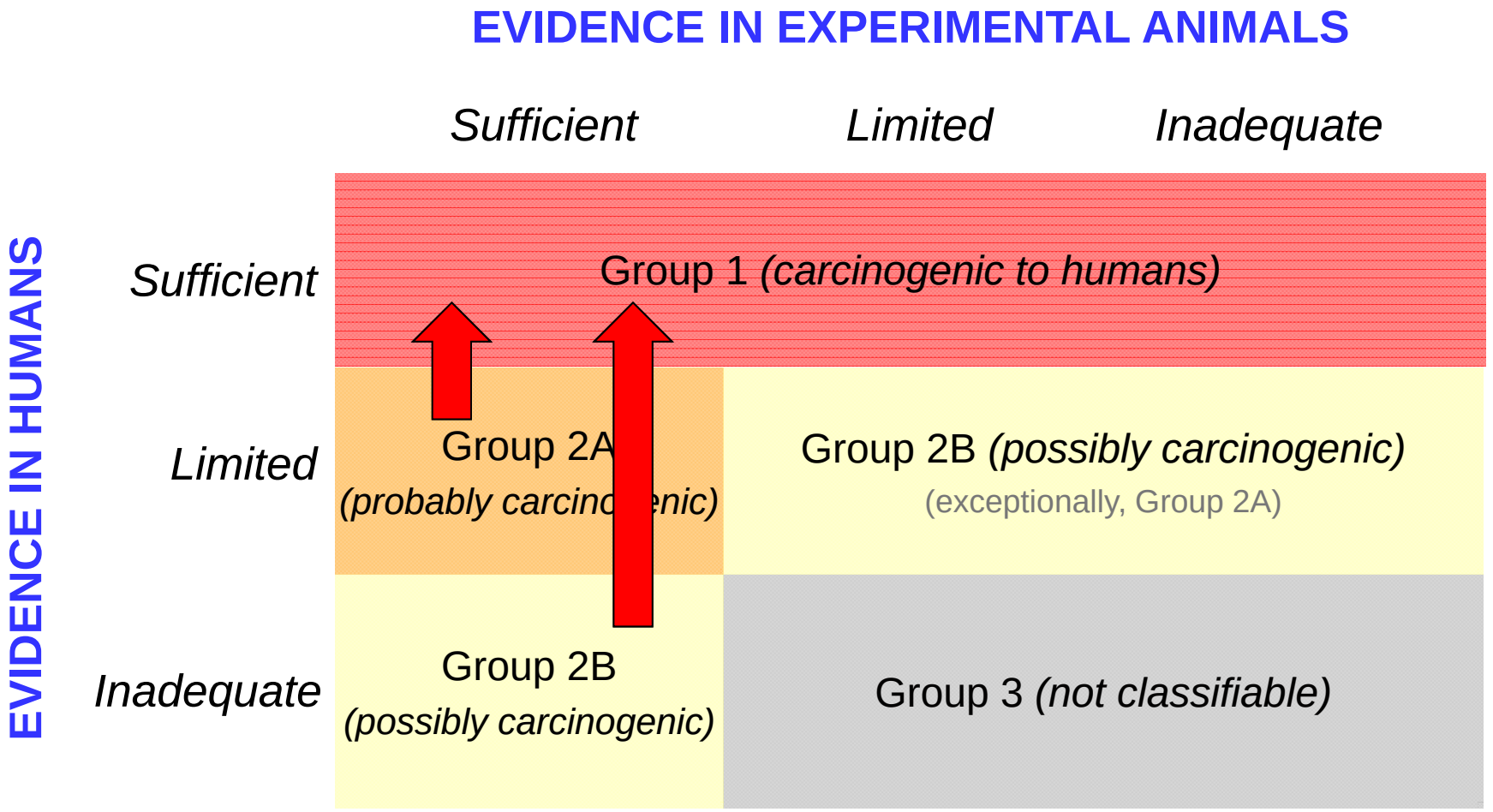
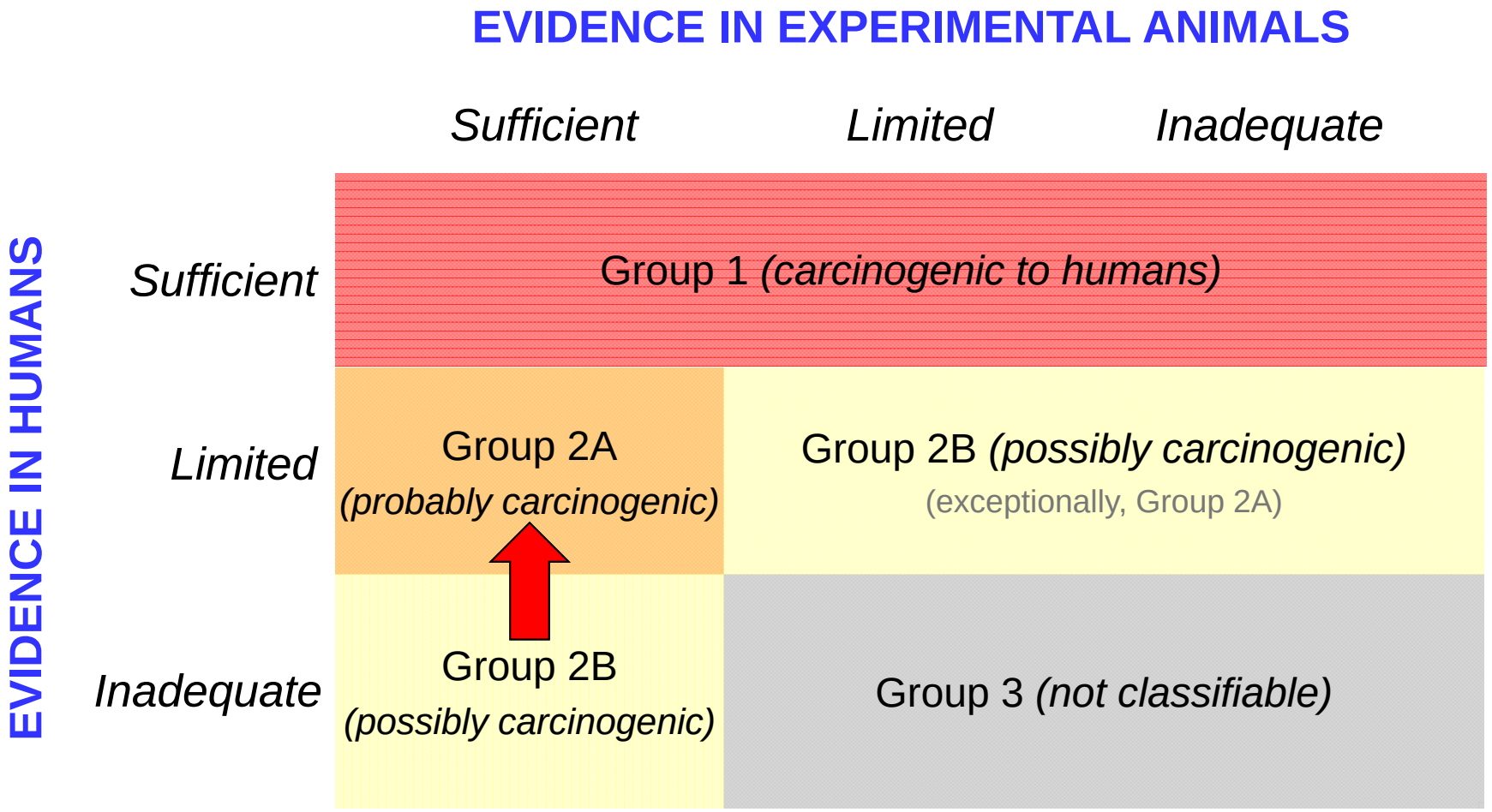
Mechanistic data are pivotal when human data are less than sufficient (Example 1)



Strong supporting evidence in exposed humans

Mechanistic data are pivotal when human data are less than sufficient (Example 2)



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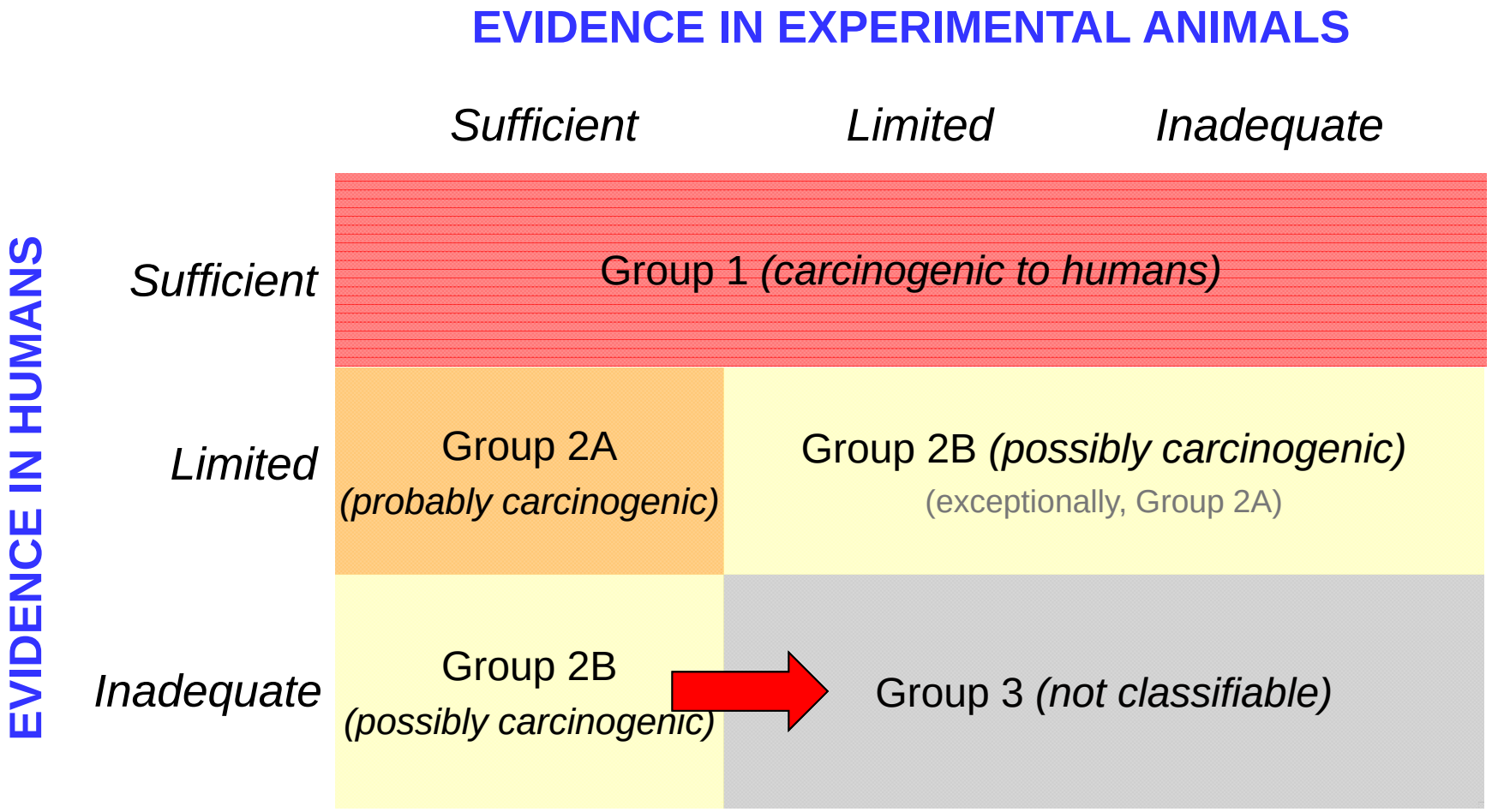
Strong evidence; mechanism also operates in humans

Mechanistic data are pivotal when human data are less than sufficient (Example 3)



Strong evidence: mechanism in animals DOES NOT operate in humans

How to identify mechanistic studies?

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:
   a. How to search systematically for relevant mechanisms?
   b. How to bring uniformity across assessments (strength- but also lack of availability- of data)?
   c. How to analyze the voluminous mechanistic database efficiently?

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Overview: IARC Monographs 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***]

IARC Secretariat:
- Recruit Working Group members and organize meeting
- Search and retrieve literature
- Assure adherence to 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:
- Sub-group review and revision
- Plenary review and evaluation

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)

Insights from Volume 100 and Advisory Groups

- The volume and complexity of mechanistic evidence is increasing
- 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
- Analysis of high-throughput/-content data (including from curated government databases) is encouraged

Sources of Mechanistic Information

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

Step 1: Identify Studies through Well-Documented Searches

- **Search for literature on each key characteristic**
- **Include results from "hand searching"**
- **Document searches and results using HAWC online tool (HAWCproject.org)**

Is Genotoxic

Actions

Description	Glyphosate and AMPA
Search Type	Search
Search Database	PubMed
Search Text	("glyphosate"[Supplementary Concept] OR "glyphosate"[All Fields]) OR ("aminomethylphosphonic acid" [Supplementary Concept] OR "aminomethylphosphonic acid"[All Fields]) AND ("Mutation"[Mesh] OR "Cytogenetic Analysis"[Mesh] OR "Mutagens"[Mesh] OR "Oncogenes"[Mesh] OR "Genetic Processes"[Mesh] OR "genomic instability"[MesH] OR chromosom* OR clastogen* OR "genetic toxicology" OR "strand break" OR "unscheduled DNA synthesis" OR "DNA damage" OR "DNA adducts" OR "SCE" OR "chromatid" OR micronucle* OR mutagen* OR "DNA repair" OR "UDS" OR "DNA fragmentation" OR "DNA cleavage")

Induces Epigenetic Alterations

Actions

Description	Glyphosate and AMPA
Search Type	Search
Search Database	PubMed
Search Text	("glyphosate"[Supplementary Concept] OR "glyphosate"[All Fields]) OR ("aminomethylphosphonic acid" [Supplementary Concept] OR "aminomethylphosphonic acid"[All Fields]) AND ("reactive oxygen species"[MeSH] OR "reactive nitrogen species"[MeSH] OR "reactive oxygen species" OR "oxygen radicals" OR "oxidative stress" [MeSH] OR oxidative OR "oxidative stress" OR "free radicals") AND ("rna"[MeSH] OR "epigenesis, genetic"[MesH] OR rna OR "rna, messenger"[MeSH] OR "rna" OR "messenger rna" OR mrna OR "histones"[MeSH] OR histones OR epigenetic OR miRNA OR methylation)

Induces Oxidative Stress

Actions

Description	Oxidative stress
Search Type	Search
Search Database	PubMed
Search Text	("glyphosate"[Supplementary Concept] OR "glyphosate"[All Fields]) OR ("aminomethylphosphonic acid" [Supplementary Concept] OR "aminomethylphosphonic acid"[All Fields]) AND ("reactive oxygen species"[MeSH] OR "reactive nitrogen species"[MeSH] OR "reactive oxygen species" OR "oxygen radicals" OR "oxidative stress" [MeSH] OR oxidative OR "oxidative stress" OR "free radicals")

HAWC HEALTH ASSESSMENT

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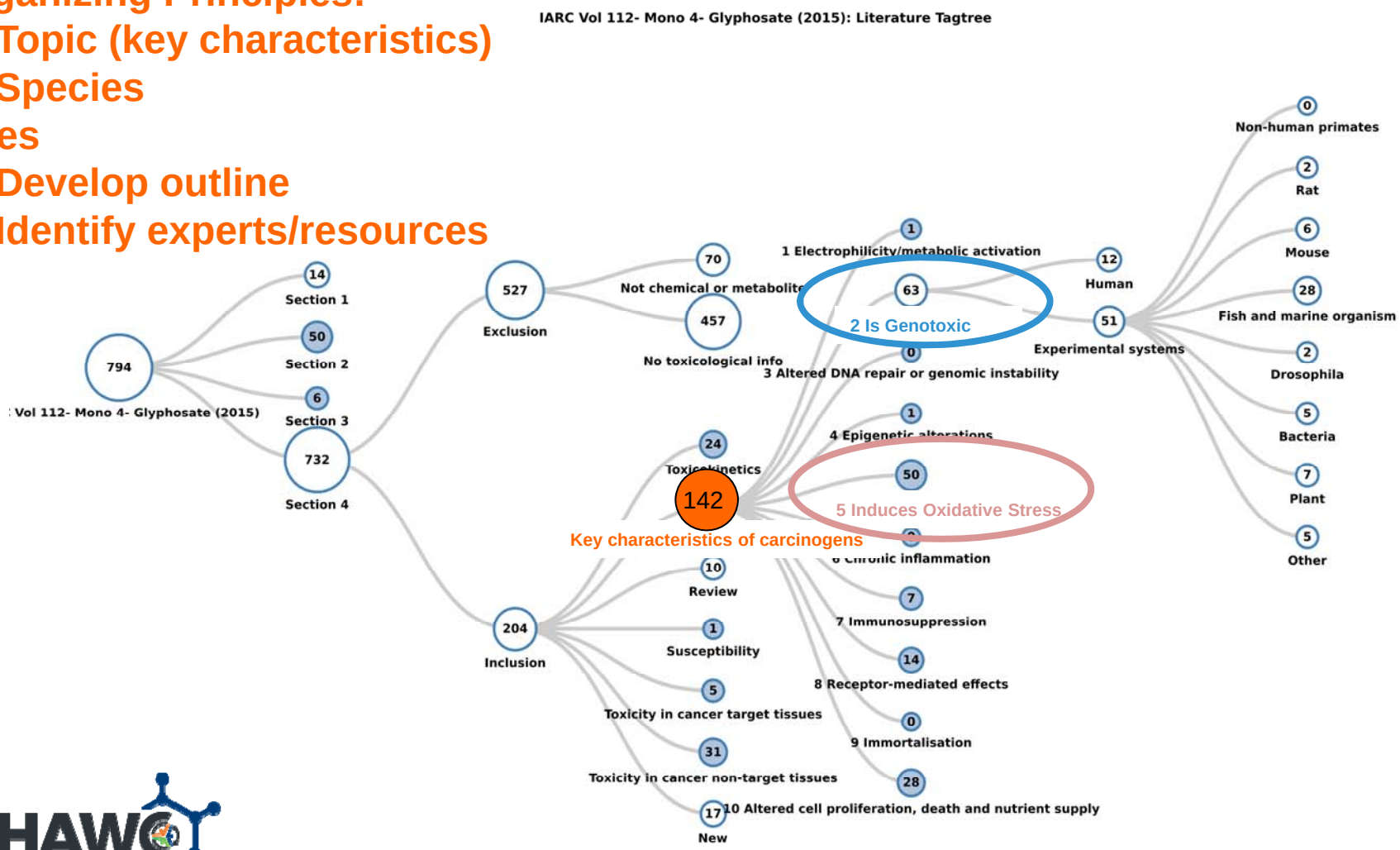
Step 2: Develop an Organized Inventory of Studies

Organizing Principles:

- **Topic (key characteristics)**
- **Species**

Uses

- **Develop outline**
- **Identify experts/resources**



Step 3: Summarize Evidence for Each Key Characteristic

Example: Glyphosate summary

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

".. 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

Can the 10 Key Characteristics Be Applied in Analyses of Tox21/ToxCast Data?

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" assay endpoints was developed

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

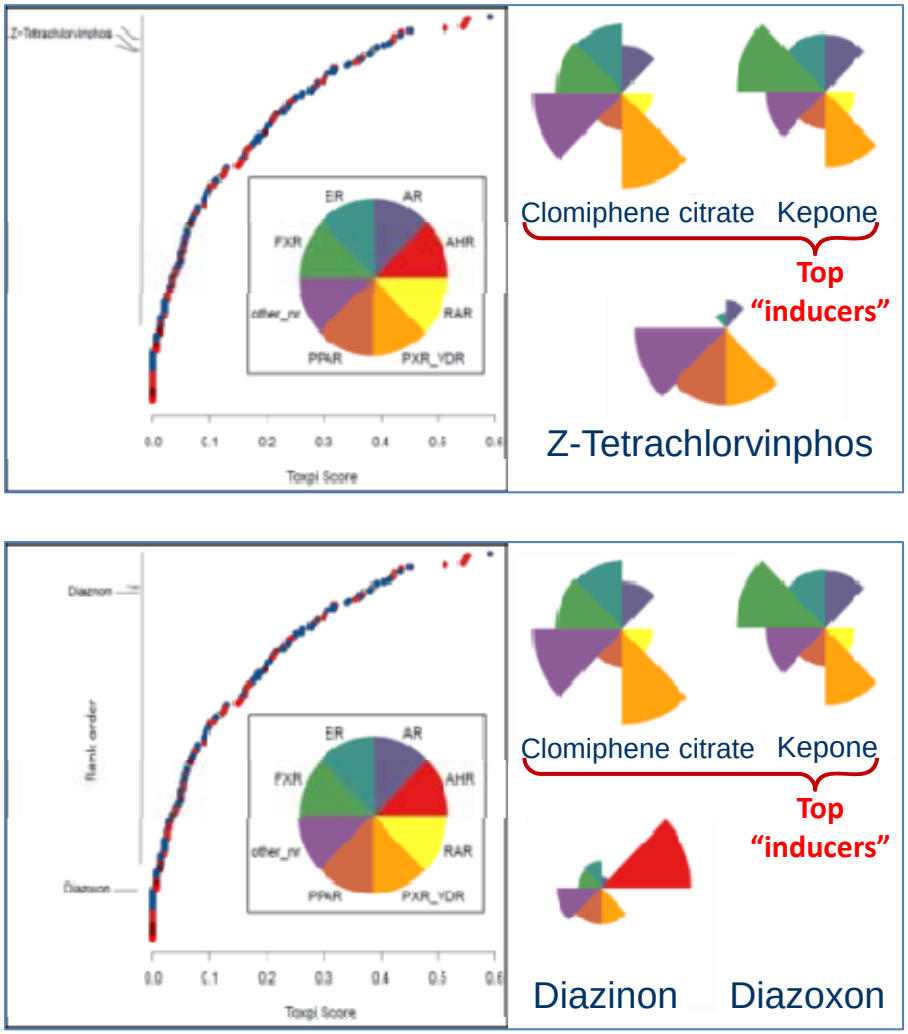
Key characteristic	1. Electrophilic or ability to undergo metabolic activation	2. Genotoxic	4. Causes Epigenetic alterations	5. Oxidative stressor	6. Induces chronic inflammation	8. Modulates receptor-mediated effects	10. Alters cell proliferation, cell death and nutrient supply
Assay Endpoints	**31 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) •Others (18) •AR (11) •PPAR (12) •ER (18) •PXR_VDR (7) •FXR (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

Use of Tox21/ToxCast Data in IARC Monographs: Examples from Volume 112

IARC-evaluated compounds that have ToxCast/Tox21 data (n=178)



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)

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 attributed to high reactivity and short half-life)

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