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.

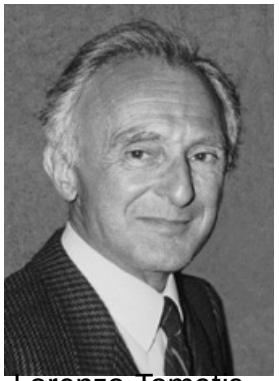
Presentation Overview

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

“The Encyclopaedia of Carcinogens”

Agents are recommended by international advisors based on:

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



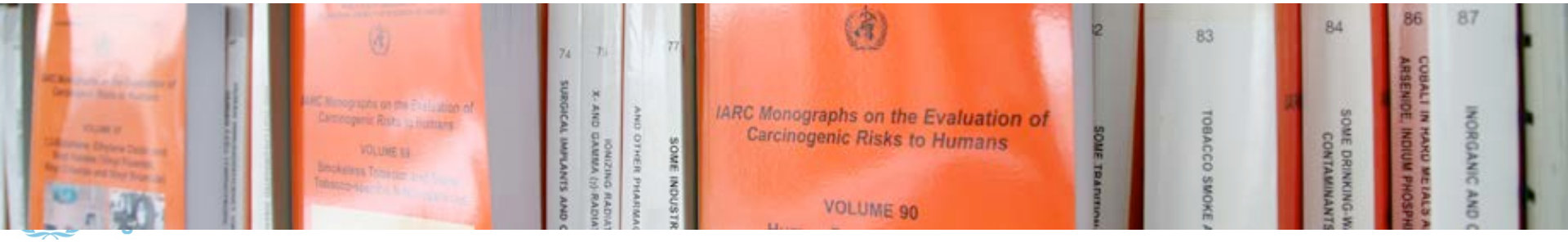
Lorenzo Tomatis
1929-2007

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 IARC Monograph Evaluations Conducted?

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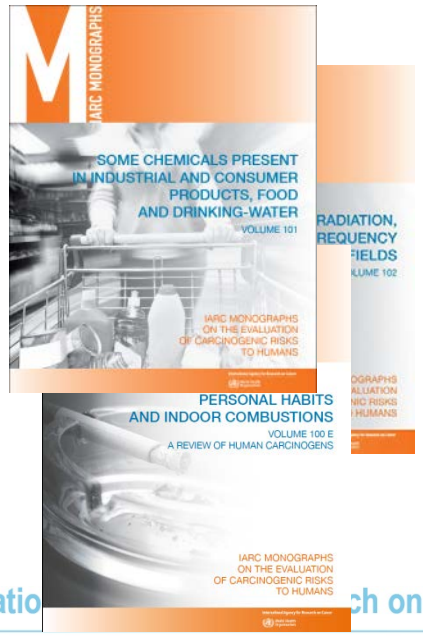
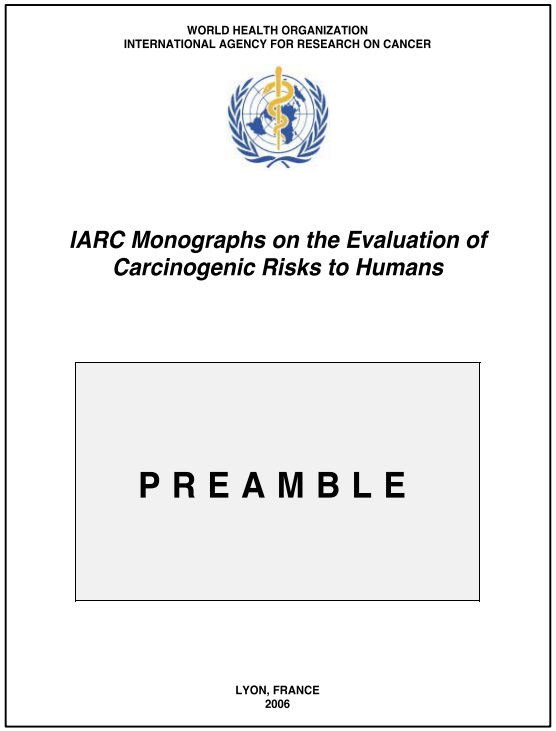
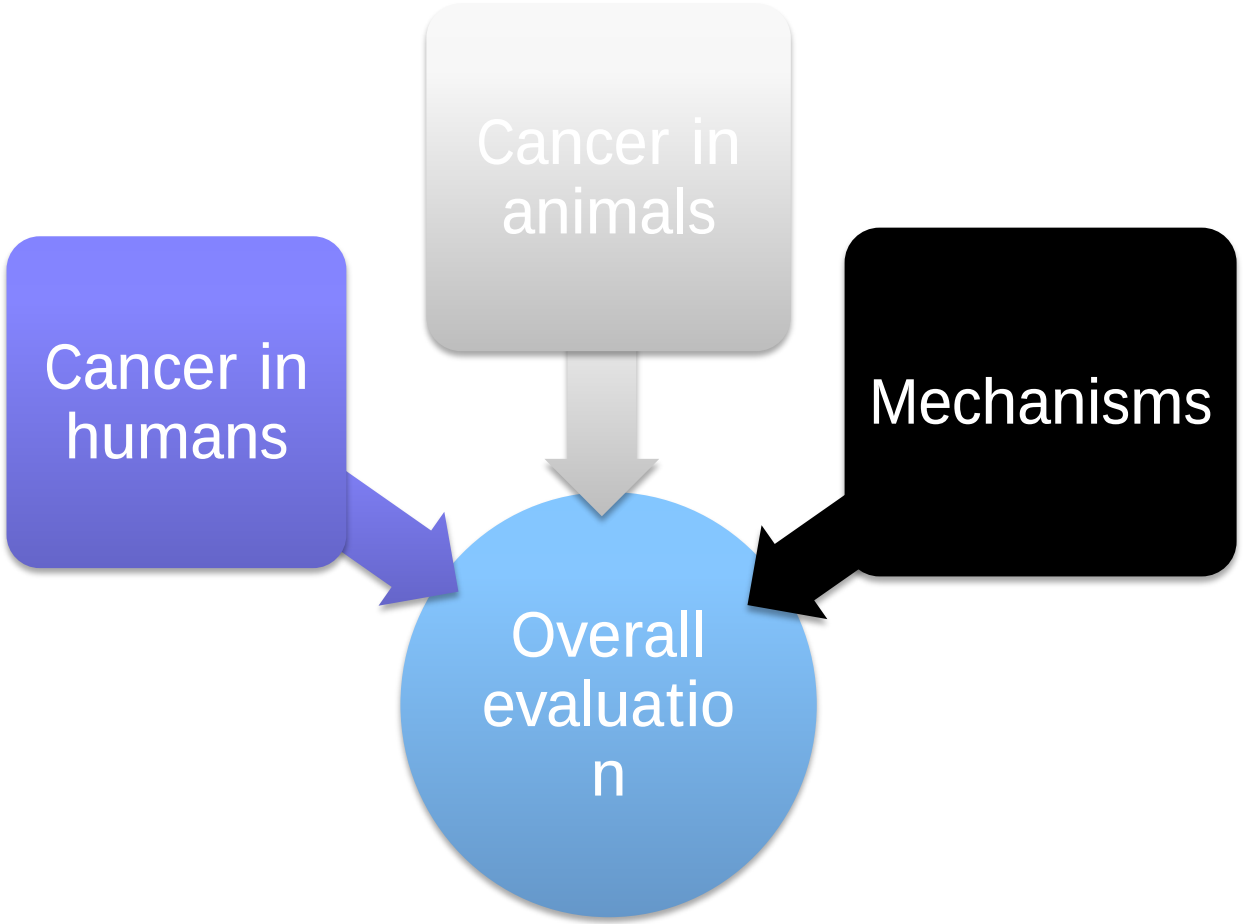




- Procedural guidelines for participant selection, conflict of interest, stakeholder involvement & meeting conduct
- Separate criteria for review of human, animal and mechanistic evidence
- Decision process for overall evaluations

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

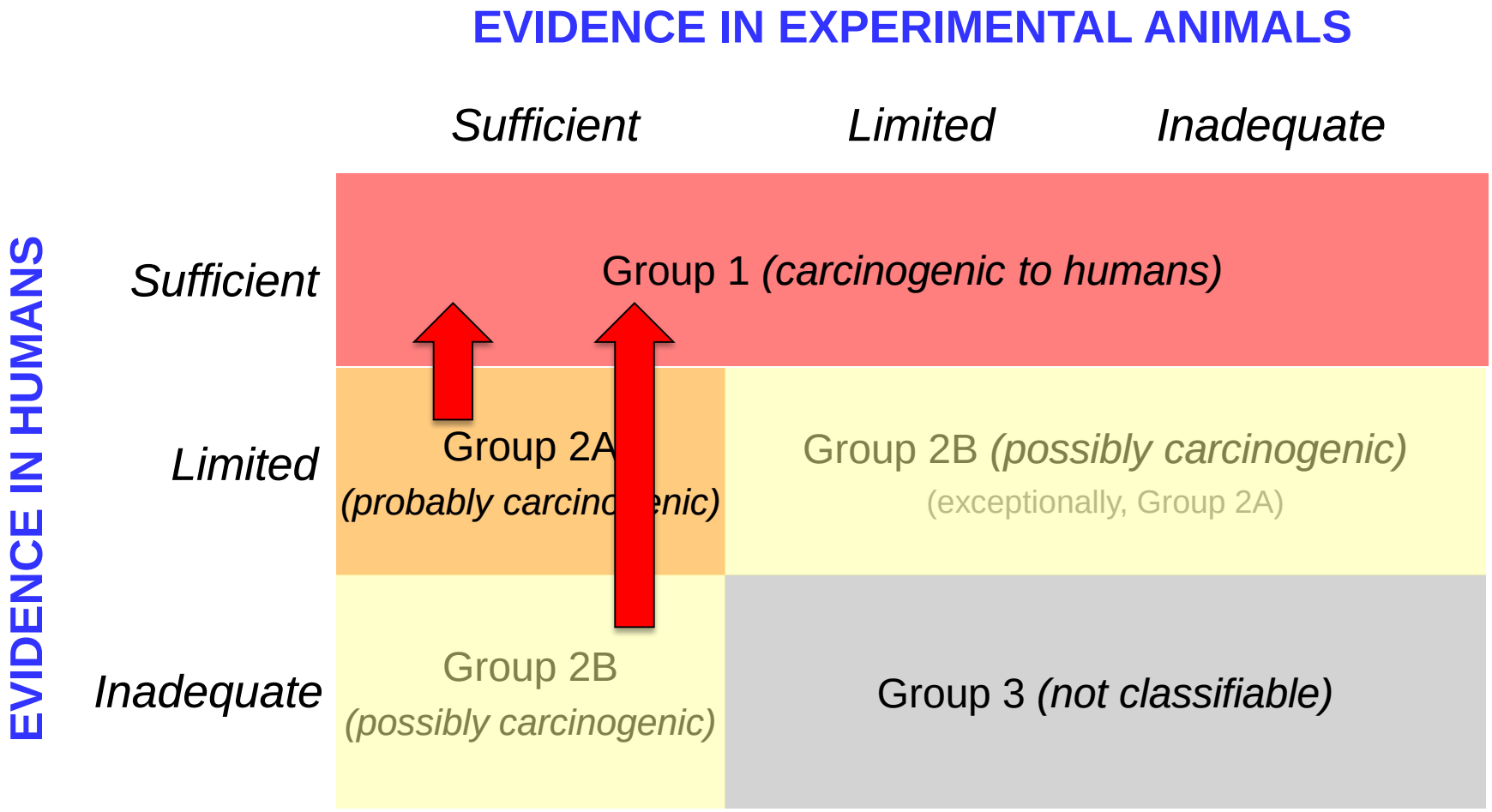
- *"Weak," "moderate," or "strong" evidence?*
- *Does this– or can it– occur in humans?*

Step 2: Integrate findings in overall evaluations

Overall evaluation

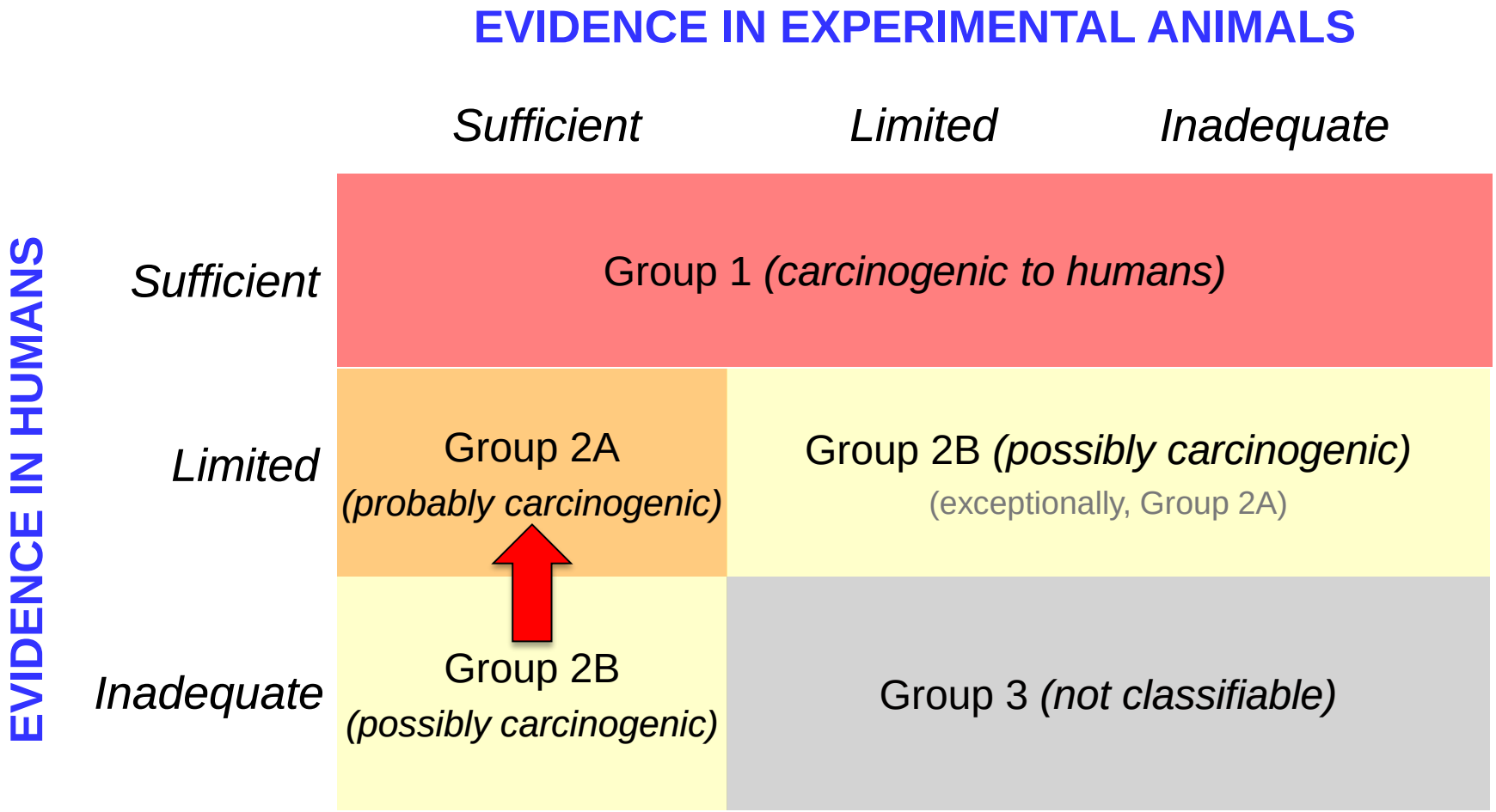
- Group 1 *Carcinogenic to humans*
- Group 2A *Probably carcinogenic to humans*
- Group 2B *Possibly carcinogenic to humans*
- Group 3 *Not classifiable as to its carcinogenicity to humans*

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



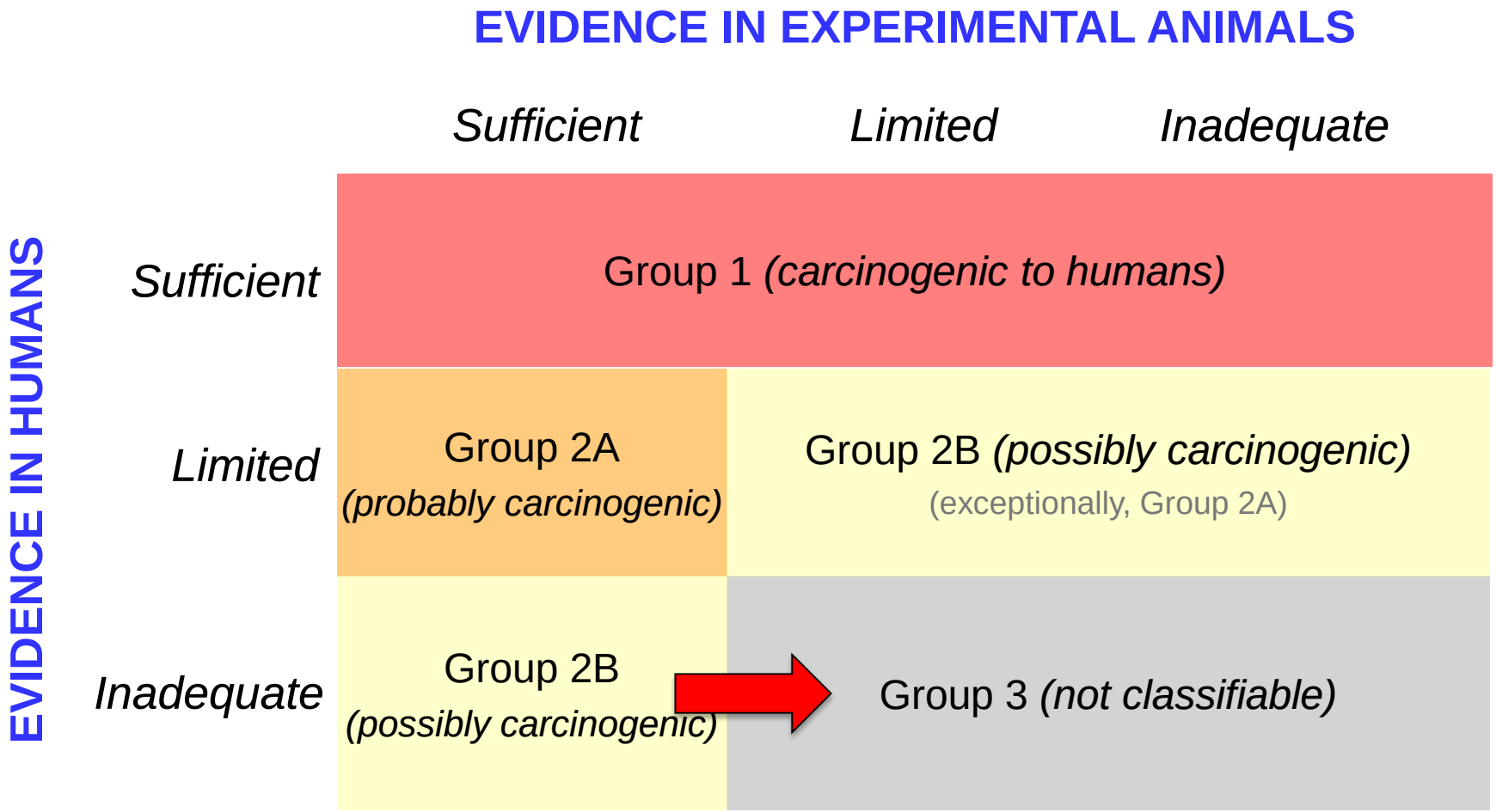
Strong supporting evidence in exposed humans

Mechanistic Data Are Pivotal When Human Data Are Not Sufficient (Example 2)



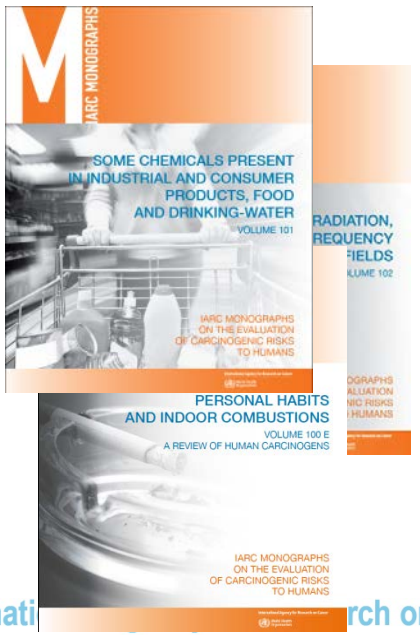
Strong evidence; mechanism also operates in humans

Mechanistic Data Are Pivotal When Human Data Are Not Sufficient (Example 3)



Strong evidence: mechanism in animals DOES NOT operate in humans

Insights from Volume 100 and Advisory Groups

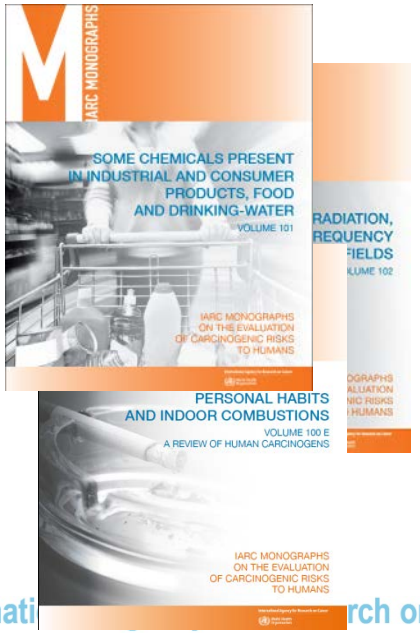


- The volume and complexity of mechanistic evidence is increasing
- Analysis of high-throughput/-content data (including from curated government databases) is encouraged
- Objective methods to identify, select and evaluate mechanistic evidence are needed
- Although not necessarily representing mechanisms 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)**

HAWC
HEALTH ASSESSMENT WORKSPACE COLLABORATIVE

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	("aminomethylphosphonic acid"[Supplementary Concept] OR "aminomethylphosphonic acid"[All Fields] OR "glyphosate"[Supplementary Concept] OR "glyphosate"[All Fields]) AND ("rna"[MeSH Terms] OR "rna"[All Fields] OR "rna, messenger"[MeSH Terms] OR "rna"[All Fields] OR "messenger rna"[All Fields] OR "mrna"[All Fields] OR "histones"[MeSH Terms] OR "histones"[All Fields] OR "epigenetic"[All Fields] OR "miRNA"[All Fields] OR "methylation"[All Fields])

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")

International Agency for Research on Cance

World Health Organization

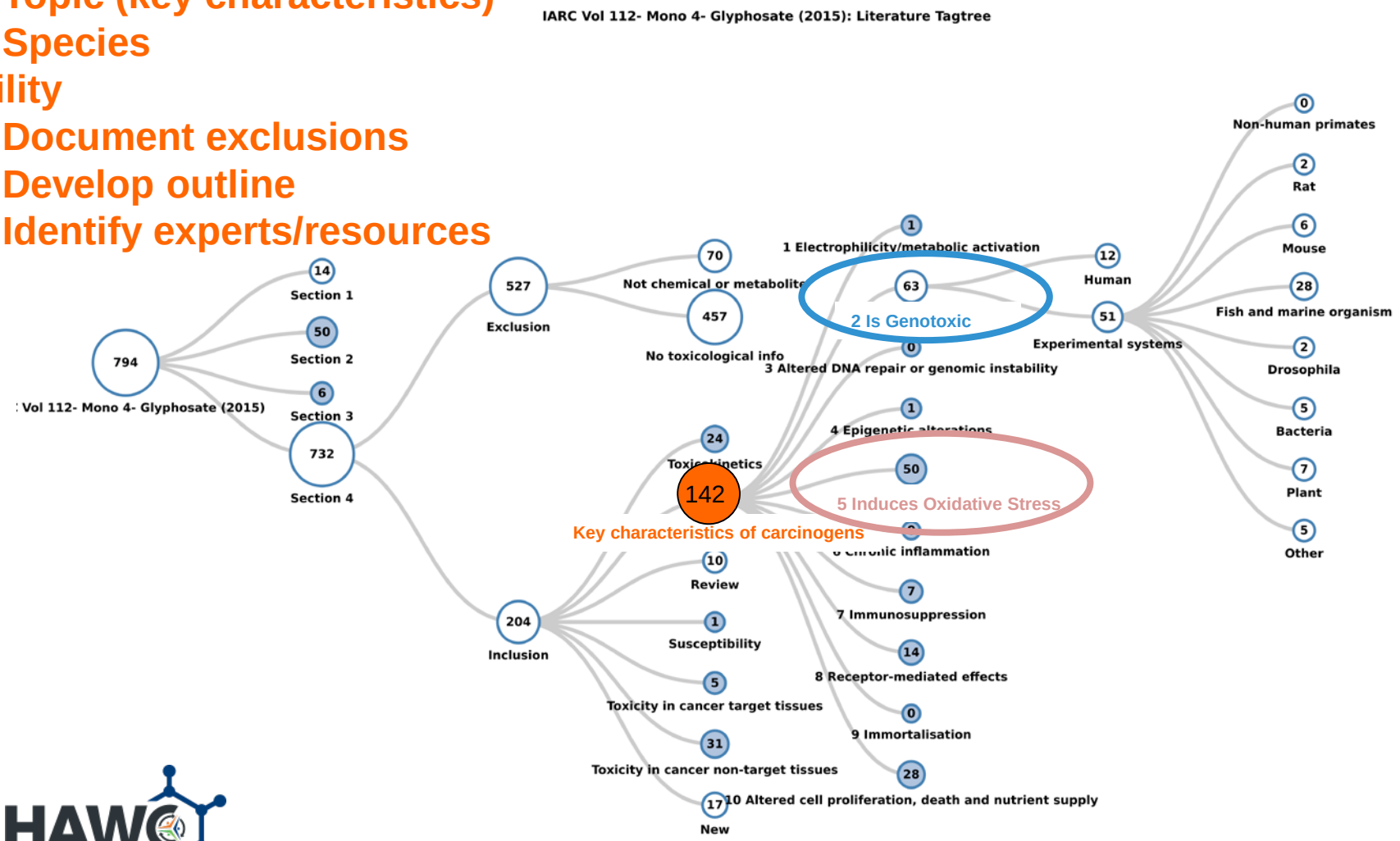
Step 2: Develop an Organized Inventory of Studies/Data

Organizing Principles:

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

Utility

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



HAWC
HEALTH ASSESSMENT WORKSPACE COLLABORATIVE

Step 2: Develop an Organized Inventory of Studies/Data

Key characteristics- endpoints and assays - Google Sheets 16/12/15 11:21

Key characteristics- endpoints and assays

File Edit View Insert Format Data Tools Add-ons Help Last edit was made on January 5 by Matthew Martin

guytonk@iarc.fr Comments Share

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	A	B	C	D	E	F	G	H
1	Characteristic	Endpoint	Assays	Related characteristic (if any) for endpoint-assay				
2	1- Electrophilicity or ability to undergo metabolic activation	Electrophilic structure (e.g., expoxide, quinone)						
3		Protein adduction	Hemoglobin adducts					
4	2- Genotoxic	DNA damage	DNA adducts					
5			DNA strand breaks					
6			DNA cross-links					
7			DNA oxidation	5				
8			Unscheduled DNA synthesis					
9			Intercalation					
10			SOS repair test					
11			Poly(ADP-ribose)polymerase induction (PADPR)					
12								
13		Mutation	Mouse spot test					
14			Mouse specific locus test					
15			Dominant lethal test					
16			Oncogene/tumor suppressor gene mutation					
17			Tk, Hprt, other gene mutation					
18			Ouabain resistance					
19			Reverse mutation					

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

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10 Key characeristic 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":

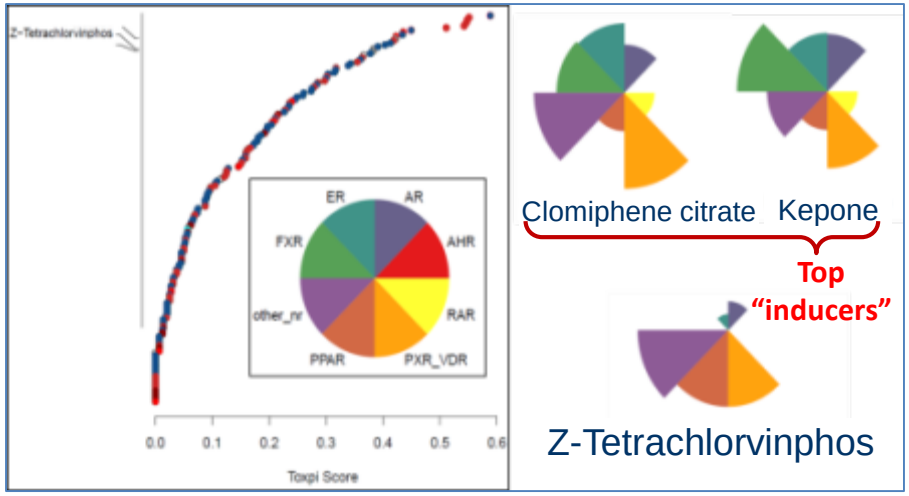
Key characteristic	1. Electrophilic or ability to undergo metabolic activation	2. Genotoxic	4. Causes Epigenetic alterations	5. Oxidative stressor	6. Induces chronic inflammation	8. Modulates receptor-mediated effects	10. Alters cell proliferation, cell death and nutrient supply
Assay Endpoints	31 assays: •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)

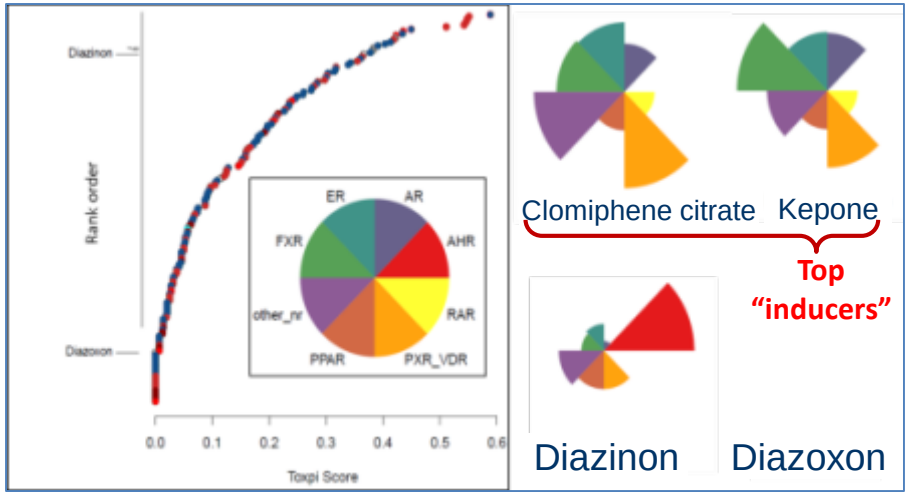
No assay coverage for these "key characteristics"

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

Step 3: Summarize Mechanistic Evidence by Key Characteristic

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 attributable to high reactivity and short half-life)

Step 3: Summarize Mechanistic Evidence by 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

Summary of Mechanistic Evidence in Recent IARC Monographs Evaluations

Agent	Human evidence	Animal evidence	Mechanistic evidence	Group
Diazinon	Limited (NHL, leukemia, lung)	Limited	Genotoxicity, oxidative stress	2A
Glyphosate	Limited (NHL)	Sufficient	Genotoxicity, oxidative stress	2A
Malathion	Limited (NHL, prostate)	Sufficient	Genotoxicity, oxidative stress, inflammation, receptor-mediated effects, and cell proliferation or death	2A
Parathion	Inadequate	Sufficient		2B
TCVP	Inadequate	Sufficient		2B
Lindane	Sufficient (NHL)	Sufficient	Immunosuppression	1
DDT	Limited (NHL, liver, testis)	Sufficient	Immunosuppression, oxidative stress, receptor-mediated effects	2A
2,4-D	Inadequate	Limited	Oxidative stress	2B

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***]

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

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

US EPA Organizers

The IARC Monographs
- Volume 100+, 112 & 113
- Working Groups

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The IARC Monographs Staff

International Agency for Research on Cancer

World Health Organization

Thank YOU– and happy holidays!