



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

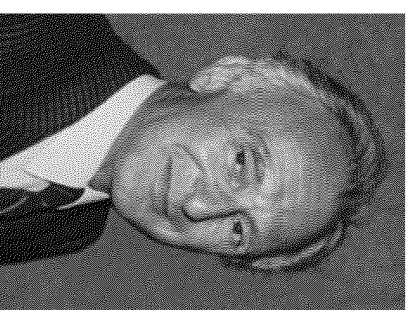
"The Encyclopaedia of Carcinogens"

Agents are recommended by international advisors based on:

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

More than 980 agents have been evaluated

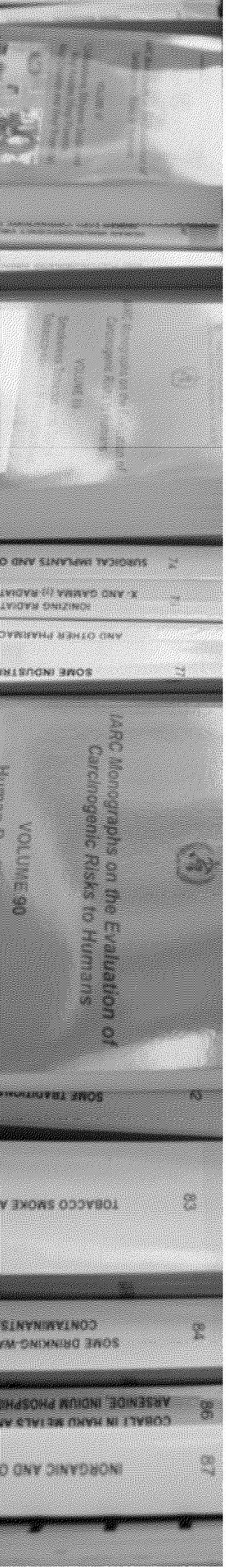
- 118 are ***carcinogenic to humans*** (Group 1)
- 75 are ***probably carcinogenic to humans*** (Group 2A)
- 287 are ***possibly carcinogenic to humans*** (Group 2B)
- 503 are ***not classifiable as to its carcinogenicity to humans*** (Group 3)
- 1 is classified as ***probably not carcinogenic to humans***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



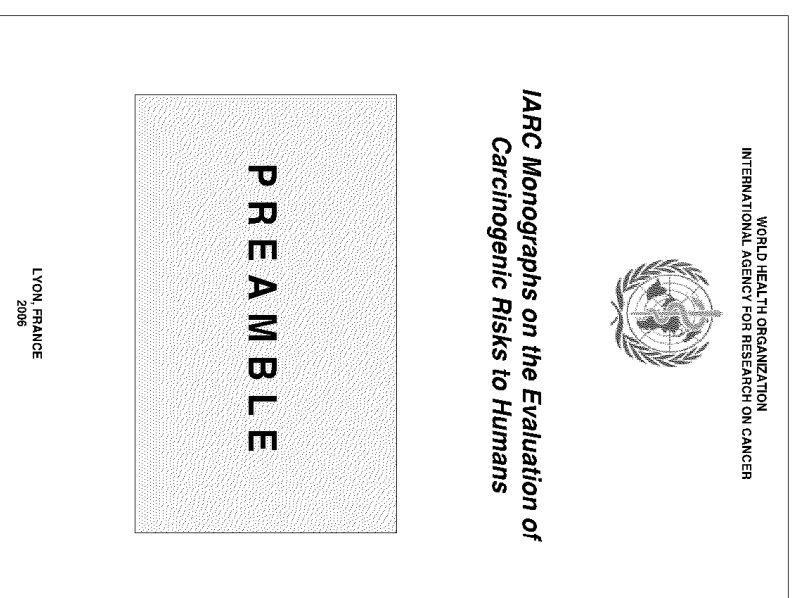
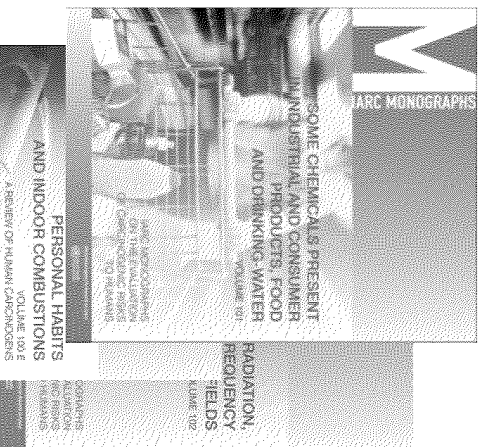
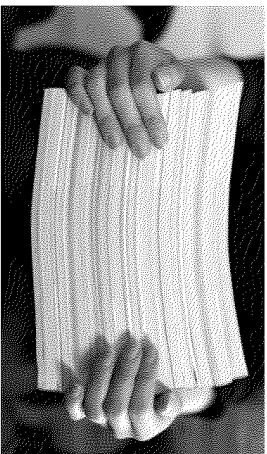
Lorenzo Tomatis
1929-2007

National and international health agencies use the *Monographs*

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



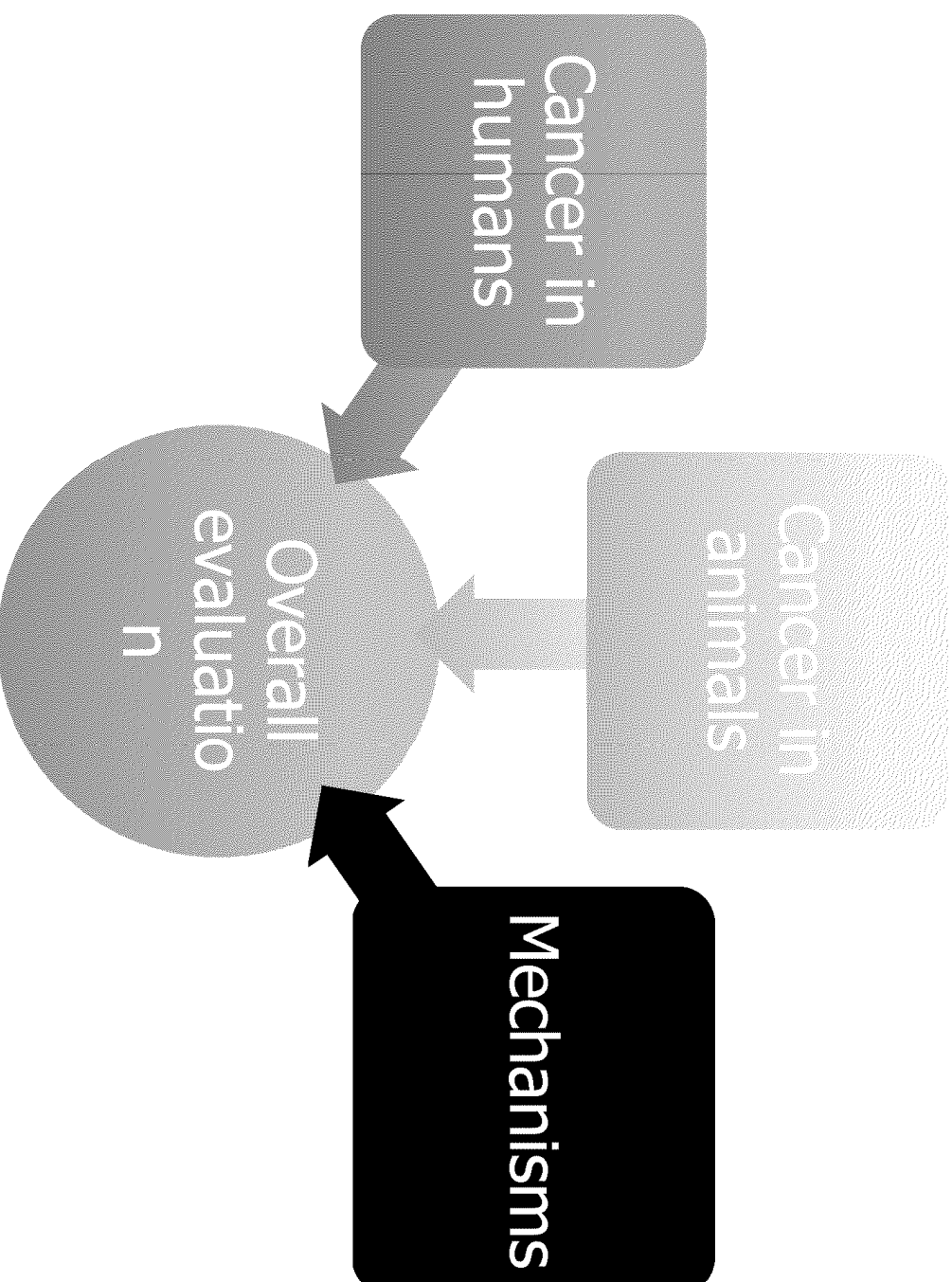
How are the IARC Monograph Evaluations Conducted?



- 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

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?

Overall evaluation

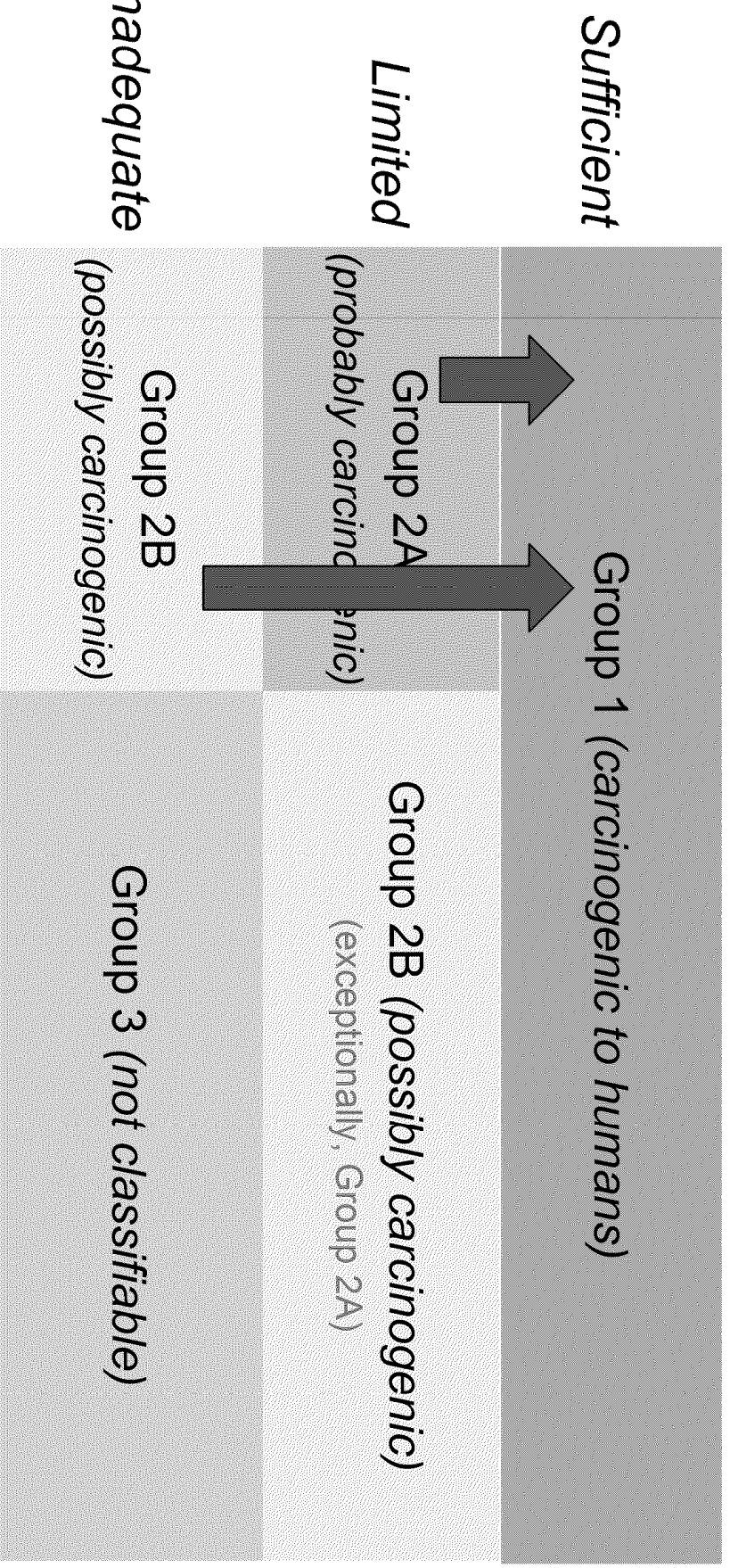
- Group 1 *Carcinogenic to humans*
- Group 2A *Probably carcinogenic to humans*
- Group 2B *Possibly carcinogenic to humans*
- Group 3 *Not classifiable as to its carcinogenicity to humans*

Step 2: Integrate findings in overall evaluations

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

EVIDENCE IN EXPERIMENTAL ANIMALS

Sufficient *Limited* *Inadequate*



EVIDENCE IN HUMANS

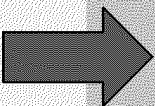
Strong supporting evidence in exposed humans

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

EVIDENCE IN EXPERIMENTAL ANIMALS

Sufficient Limited Inadequate

EVIDENCE IN HUMANS	EVIDENCE IN EXPERIMENTAL ANIMALS		
	Sufficient	Limited	Inadequate
Sufficient	Group 1 (carcinogenic to humans)		
Limited	Group 2A (probably carcinogenic)	Group 2B (possibly carcinogenic) (exceptionally, Group 2A)	
Inadequate	Group 2B (possibly carcinogenic)	Group 3 (not classifiable)	



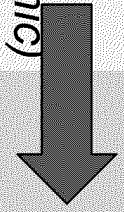
Strong evidence; mechanism also operates in humans

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

EVIDENCE IN EXPERIMENTAL ANIMALS

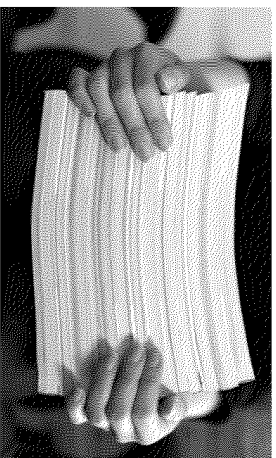
Sufficient Limited Inadequate

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



Strong evidence: mechanism in animals
DOES NOT operate in humans

How to identify mechanistic studies?

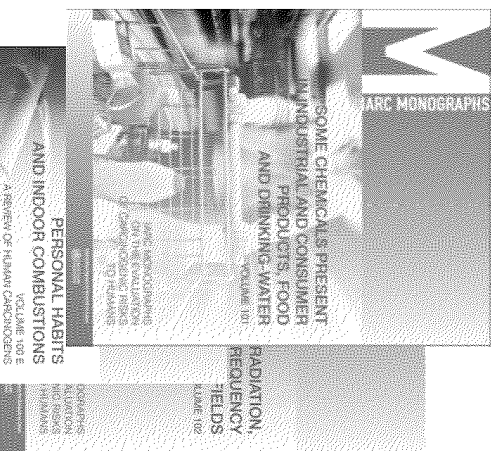


Considerations:

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?



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 Col form posted

List of

Participants

announced (Jan. 2015)

The Lancet

Oncology

publication (March 2015)

References

shared with

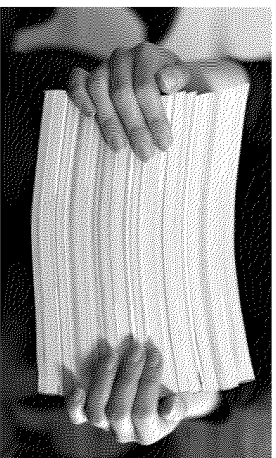
health agencies (April 2015)

Glyphosate

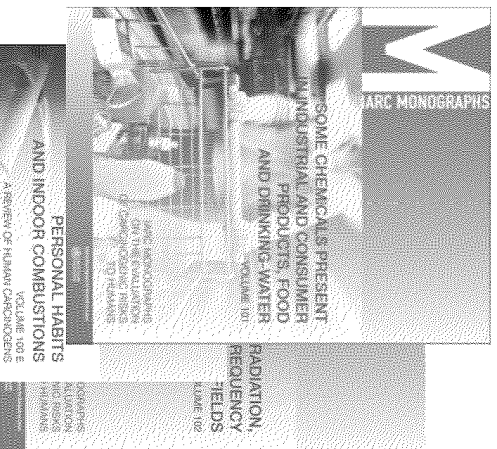
Monograph

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

Description	Glyphosate and AMPA	Actions -
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 "chromosome" 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

Description	Glyphosate and AMPA	Actions -
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 "apoptosis genetic"[Mesh] OR "rna" OR "rna, messenger"[Mesh] OR "ma" OR "messenger" OR "mma" OR "histones"[Mesh] OR "histones OR epigenetic" OR "miRNA" OR "methylation")	

Induces Oxidative Stress

Description	Oxidative stress	Actions -
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")	



Step 2: Develop an Organized Inventory of Studies

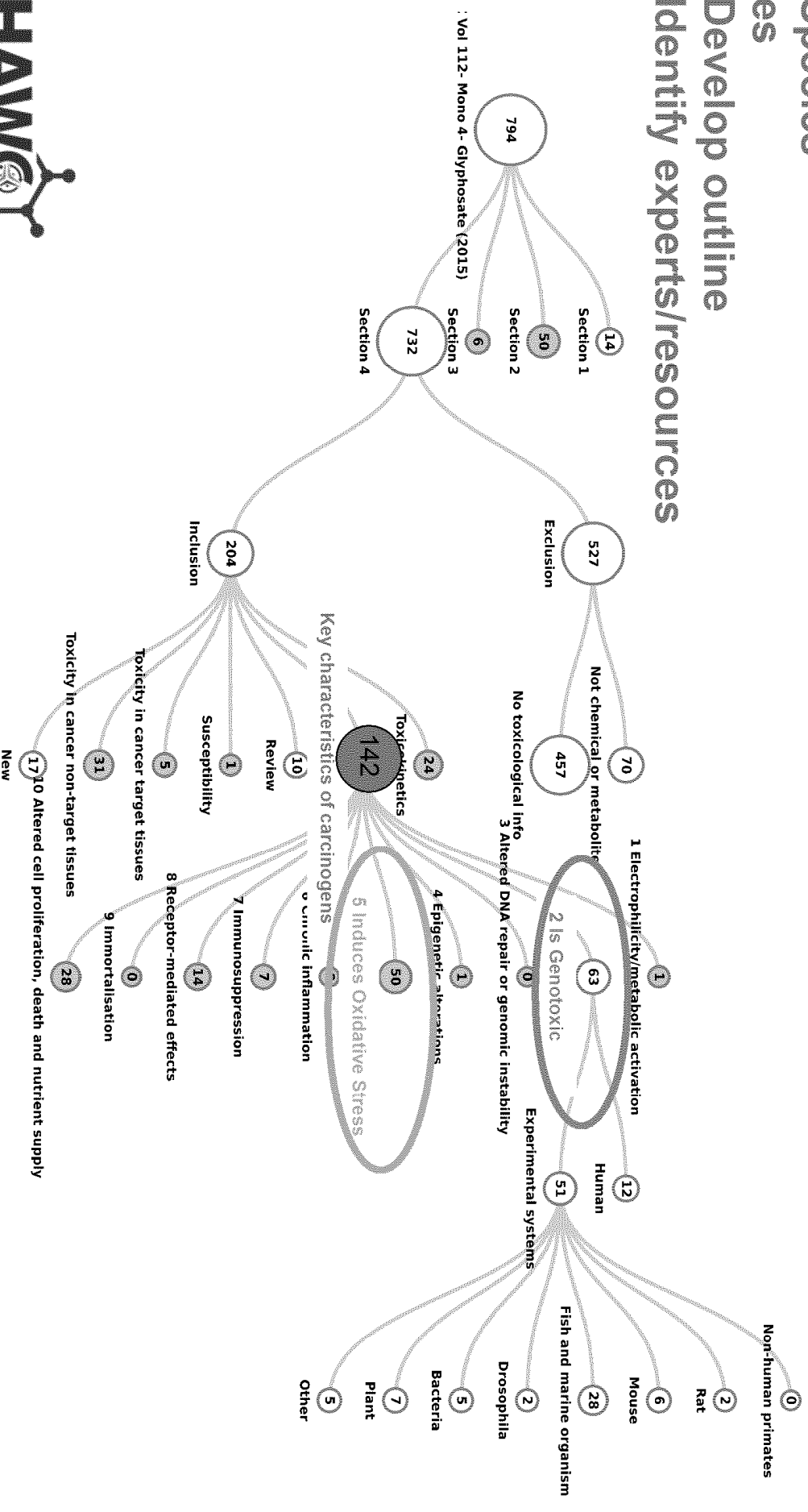
Organizing Principles:

- Topic (key characteristics)
- Species

Uses

- Develop outline
- Identify experts/resources

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



Step 3: Summarize Evidence for Each Key Characteristic

Example: Glyphosate summary

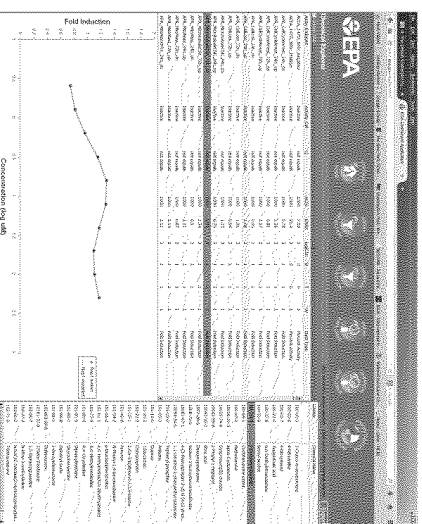
Characteristic	Strength of evidence for glyphosate	Does this– or can it– operate in humans?
1. Is Electrophilic or Can Be Metabolically Activated	Not electrophilic	
2. Is Genotoxic	Strong	Can operate in humans
3. Alters DNA Repair or Causes Genomic Instability	No data	
4. Induces Epigenetic Alterations	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”**

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

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)

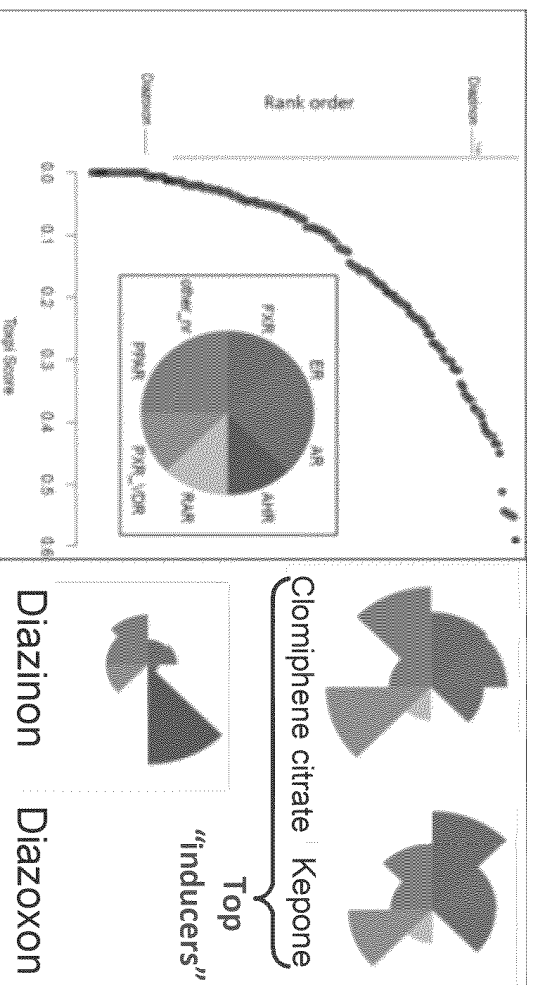
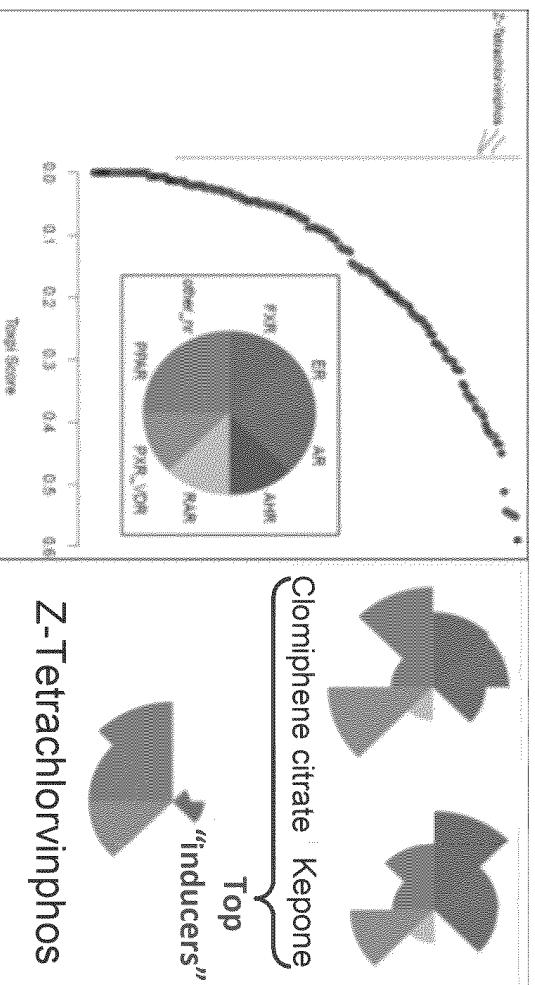
No assay coverage for these “key characteristics”



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