The key characteristics approach to evaluating mechanistic data in hazard identification and risk assessment

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Conflict of Interest Statement

- I am retained as a consultant and potential expert witness in U.S. litigation involving chemical exposures and disease outcomes, including cancer, on behalf of plaintiffs.
- I have no formal association with IARC, US EPA or CalEPA, but have an ongoing contract with OEHHA (Cal EPA) to further develop the key characteristics.
- The views expressed are solely my own.

Summary of today's talk

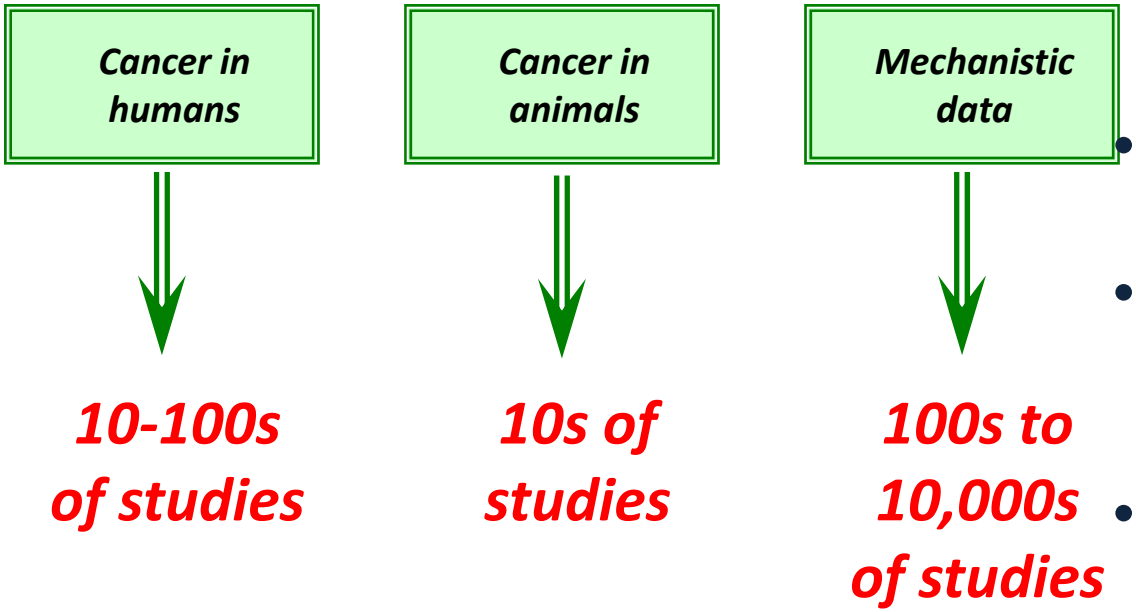
- Scientific findings providing insights into cancer mechanisms play an increasingly important role in carcinogen hazard identification
- **The key characteristics (KCs) of human carcinogens provide the basis for a knowledge-based approach to evaluating mechanistic data rather than a hypothesis-based one like MOA/AOP**
- Recent IARC Monograph, EPA, CalEPA and NTP evaluations have illustrated the applicability of the KC approach
- May be compatible with HT assays, but need to develop new ones based on characteristics and hallmarks. Same for biomarkers.
- Key characteristics for other forms of toxicity are being developed
- KCs could be used in data-science approach to prioritorize chemicals for further evaluation

Mechanistic Data: *Challenges*

IARC Monographs
Volume 100

- Different human carcinogens may operate through distinct mechanisms
- Many human carcinogens act via multiple mechanisms
- There was no broadly accepted, systematic method for evaluating mechanistic data to support cancer hazard identification

So Many Studies, So Little Time…

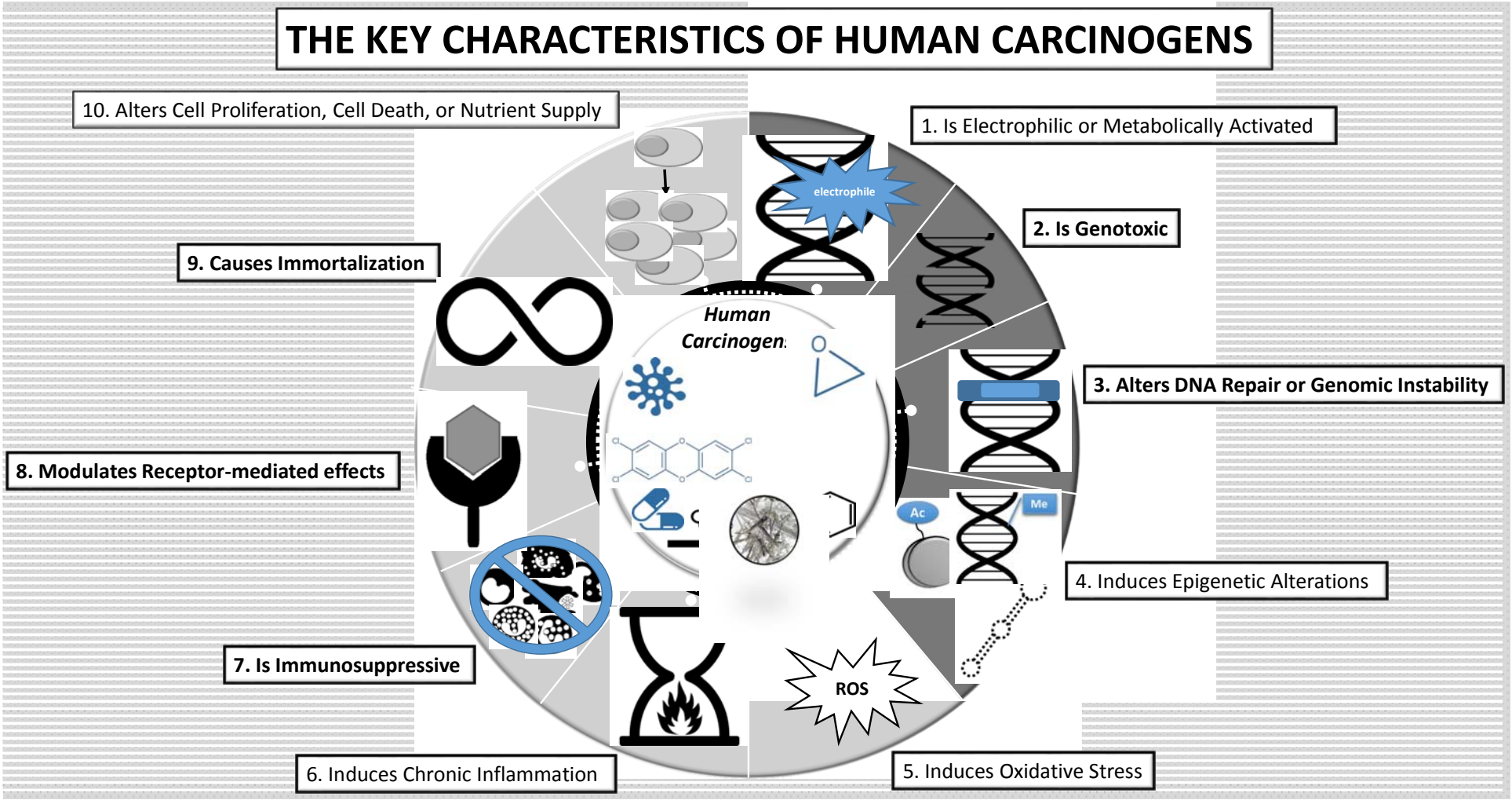


- How to search systematically for relevant mechanisms?
- How to bring uniformity across assessments?
- How to analyze the voluminous mechanistic database efficiently?
- How to avoid bias towards favored mechanisms

KCs resulted from a large collaboration

- **IARC**: Kathryn Z. Guyton, Robert Baan and Kurt Straif
- **US EPA**: Catherine Gibbons, Jason Fritz, David DeMarini, Jane Caldwell, Robert Kavlock, Vincent Cogliano
- **NTP**: John Bucher **FDA**: Frederick Beland
- **Academia**: Ivan Rusyn, Paul F. Lambert, Stephen S. Hecht, Bernard W. Stewart, Weihsueh Chiu, Denis Corpet, Martin van den Berg, Matthew Ross, David Christiani
- **Consultant**: Christopher Portier
- **Acknowledgements:** Michele La Merrill for discussion and support from OEHHA, Research Translation Core of NIEHS SRP grant P42ES004705 and travel awards from IARC.

THE KEY CHARACTERISTICS OF HUMAN CARCINOGENS



Guyton KZ, Rieswijk L, et al., Chemical Res. In Toxicology, December 6, 2018

INTEGRATION OF THE KCs WITH HALLMARKS

Characteristics 1,2,4 and 8 can influence all Hallmarks; 7=7, 3=1, 6=9

Key Characteristics

1. Is electrophilic or can be metabolically activated

2. Is genotoxic

3. Alters DNA repair or causes genomic instability

4. Induces epigenetic alterations

5. Induces oxidative stress

6. Induces chronic inflammation

7. Is immunosuppressive

8. Modulates receptor-mediated effects

9. Causes immortalization

10. Alters cell proliferation, cell death, or nutrient supply

Hallmarks

1. Genetic Instability

2. Sustained Proliferative Signalling

3. Evasion of Anti-growth Signalling

4. Resistance to Cell Death

5. Replicative Immortality

6. Dysregulated Metabolism

7. Immune System Evasion

8. Angiogenesis

9. Inflammation

10. Tissue Invasion and Metastasis

PLUS - Tumor Microenvironment

MT Smith, UCB Dec 2018

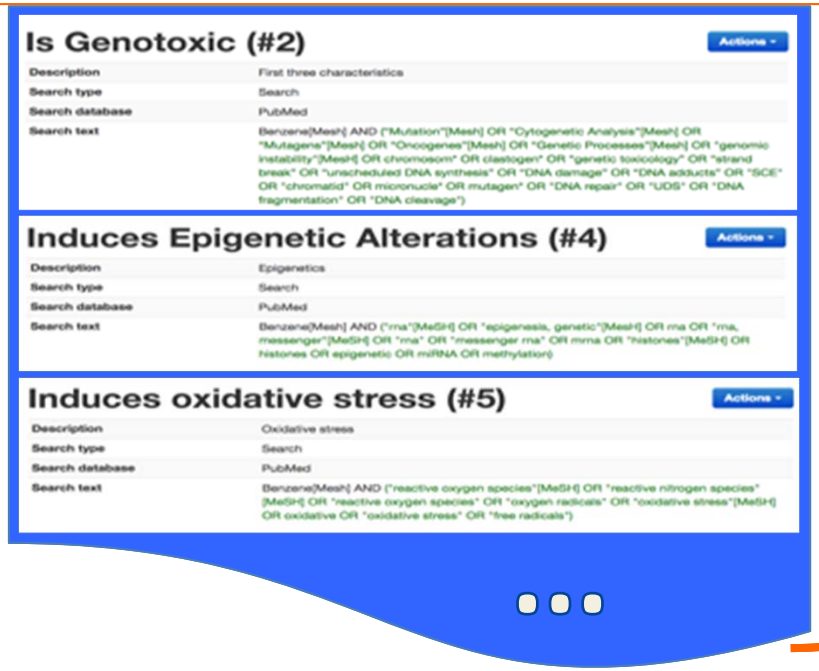
KCs act by disrupting Hallmark processes – Conclusion of Working Group convened in Berkeley, August 21-22, 2018

Applications of the KCs

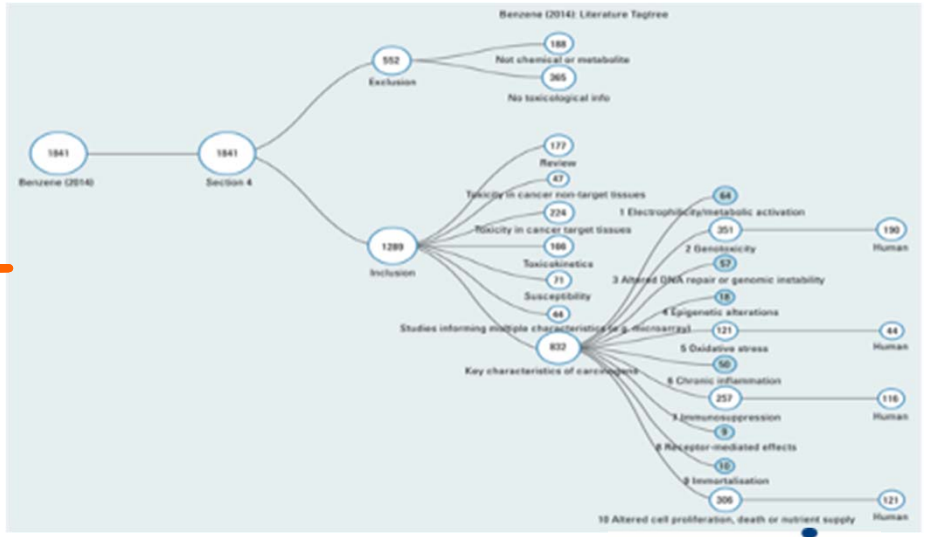
- Searching the literature – Set of MeSH terms developed – Facilitate systematic review
- Identify data gaps
- Development of MOA/AOP or networks
- Improve predictive toxicology
- Better understanding of cumulative risk

Systematic Approach
Using Key Characteristics of Carcinogens

Targeted searches for each key characteristic



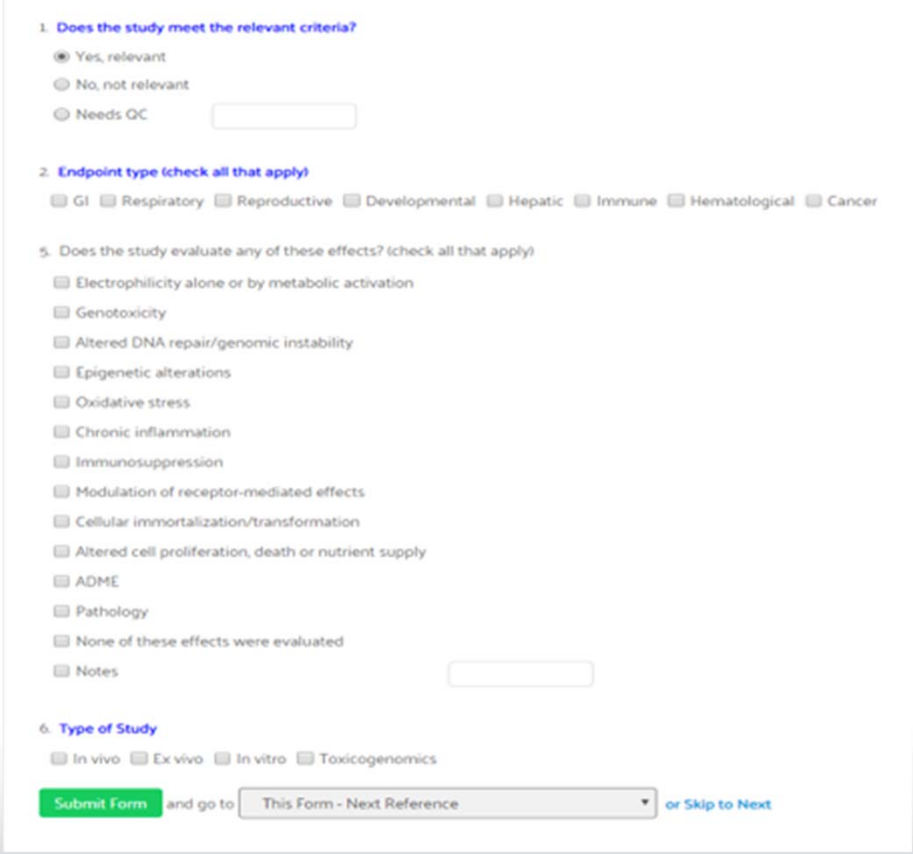
Organize results by key characteristics, species, etc



HAWC
HEALTH ASSESSMENT WORKSPACE COLLABORATIVE

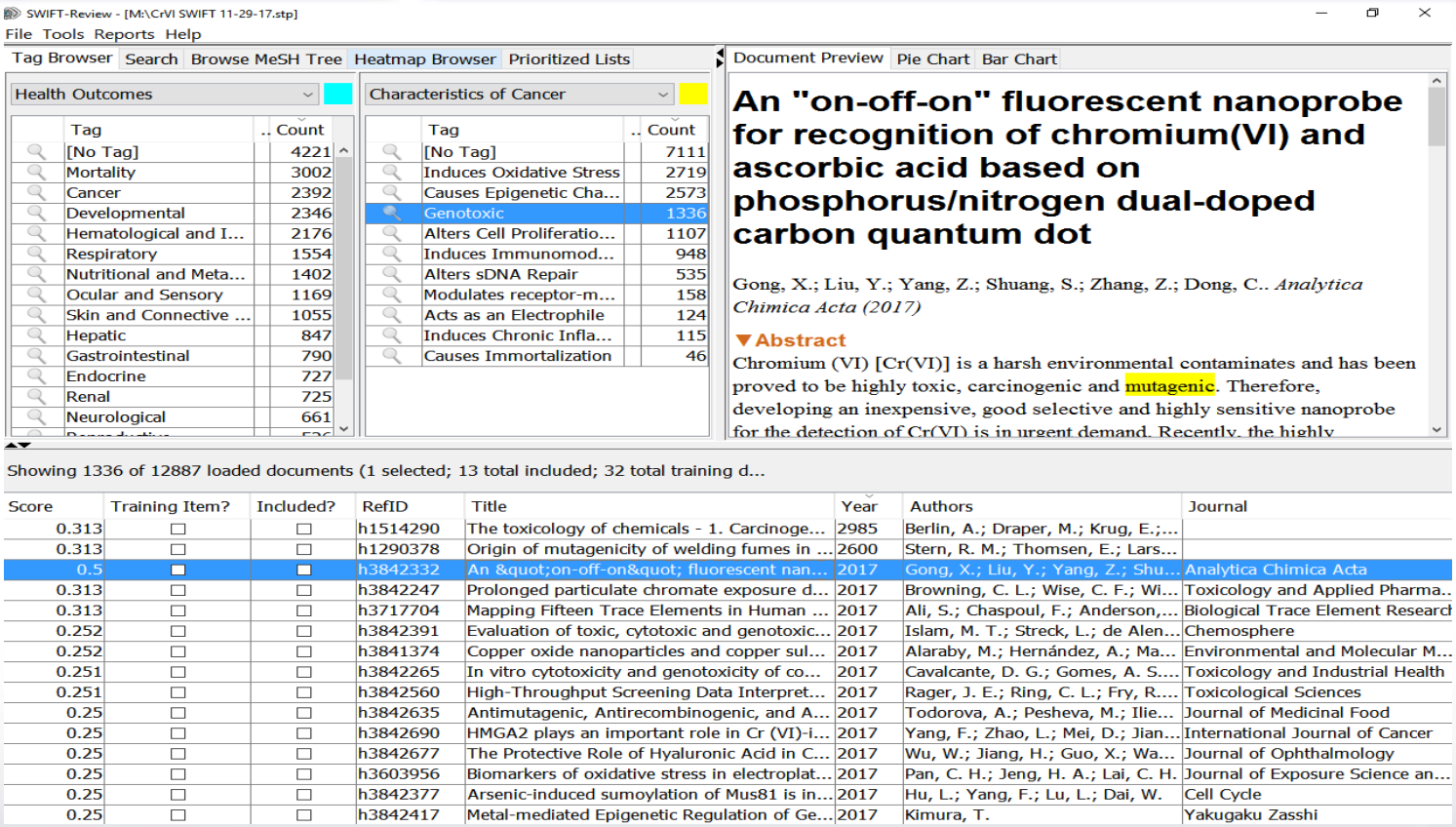
Smith MT, Guyton KZ, Gibbons CF, Fritz JM et al.. *Env Health Persp.*, *124(6):713-21*

10 KCs in Literature Screening (e.g., Distiller)



Slide from Catherine Gibbons, EPA

10 KCs in automated literature sorting and screening (SWIFT)



Slide from Catherine Gibbons, EPA

Application of the KCs at IARC

Use the KCs to:

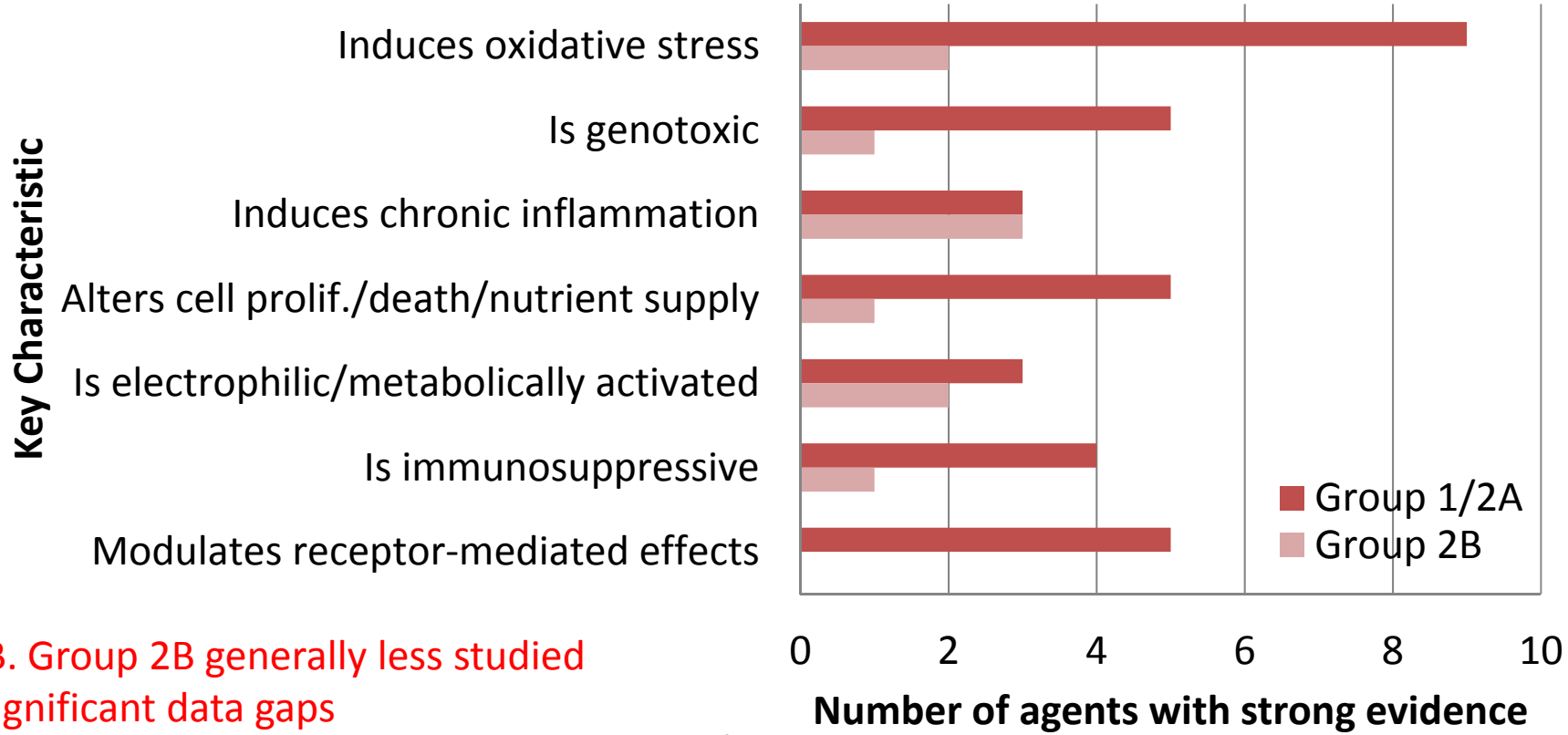
- Identify the relevant mechanistic information
- Screen and organize the search results
- Evaluate quality of the identified studies
- Summarize the evidence for each KC as strong, moderate or weak and determine if it operates in humans or human in vitro systems

Use of KCs in Recent IARC Monographs Evaluations

Agent	Group	Cancer in humans	Cancer in animals	Strong mechanistic evidence (key characteristic)
Penta-chlorophenol	1	**Sufficient**	Sufficient	Is metabolically activated, is genotoxic, induces oxidative stress, modulates receptor-mediate effects, alters cell proliferation or death (1, 2, 5, 6, 8, 10)
Welding fumes	1	**Sufficient**	Sufficient	Are immunosuppressive, induce chronic inflammation (6, 7)
DDT	2A	**Limited**	**Sufficient**	Modulates receptor-mediated effects, is immunosuppressive, induces oxidative stress (5,7,8)
Dimethyl-formamide	2A	**Limited**	**Sufficient**	Is metabolically activated, induces oxidative stress, alters cell proliferation (1, 5, 10)
Tetrabromo-bisphenol A	**2A***	Inadequate	**Sufficient**	**Modulates receptor-mediated effects, is immunosuppressive, induces oxidative stress (5, 7, 8)**
Tetrachloro-azobenzene	**2A***	Inadequate	**Sufficient**	**Induces oxidative stress, is immunosuppressive, modulates receptor-mediated effects (6, 8, 10)**
ITO, melamine	2B	Inadequate	**Sufficient**	Induces chronic inflammation (8)
Parathion, TCP	2B	Inadequate	**Sufficient**	

*Overall evaluation upgraded to Group 2A with supporting evidence from other relevant data

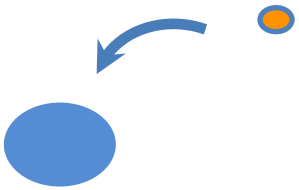
Key Characteristics with Strong Evidence across Multiple Evaluations (IARC Monographs Vol. 112-119)



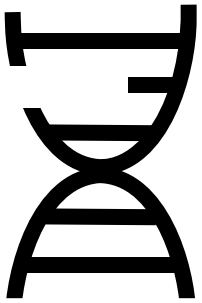
N.B. Group 2B generally less studied – significant data gaps

Strong Evidence of 5 Key Characteristics for Sb^{III}

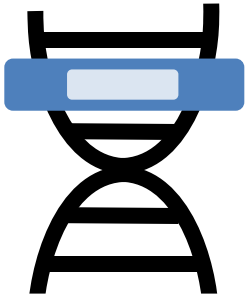
Electrophilic



Genotoxic

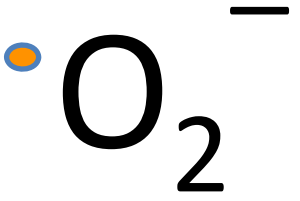


⬇ DNA repair



➡ Epigenetic alteration

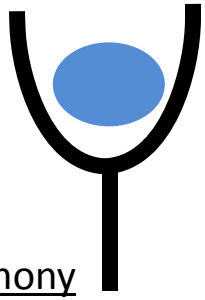
⬆ Oxidative stress



➡ Chronic inflammation

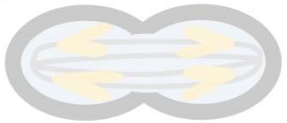
⬆⬇ Immune response

➡ Receptor-mediated effects



➡ Cell immortalization

⬆ Cell proliferation,



⬇ cell death,

or alter nutrient supply

"Report on Carcinogens Monograph on Antimony Trioxide" https://ntp.niehs.nih.gov/pubhealth/roc/listings/antimonyt/index.html

Applications of the KCs

- Searching the literature – Set of MeSH terms developed – Facilitate systematic review
- Identify data gaps
- Development of MOA/AOP or networks
- Improve predictive toxicology
- Better understanding of cumulative risk

Use of the KCs by the NTP Report on Carcinogens

Table 6-4. Possible modes of carcinogenic action for haloacetic acids and the 10 characteristics of carcinogens

Characteristic(s) of carcinogens	Mode of action	Key events
Electrophilicity	Irreversible binding to macromolecules	1. Haloacetic acids have an electrophilic structure that can react with peptides, proteins, or DNA to form adducts. 2. Protein or DNA adducts result in altered activity or DNA damage that advances acquisition of multiple critical traits contributing to carcinogenesis.
Altered nutrient supply, electrophilicity, induction of oxidative stress	Reprogramming cellular energy metabolism (inhibition of pyruvate dehydrogenase kinase (PDK)	1. Haloacetic acids inhibition of PDK increases pyruvate dehydrogenase complex activity and oxidative metabolism. 2. Increase in oxidative metabolism leads to an increase in reactive oxygen species (ROS) and oxidative stress. 3. Oxidative stress leads to acquisition of multiple, critical traits contributing to carcinogenesis.
Altered nutrient supply, electrophilicity, induction of oxidative stress	Inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	1. Haloacetic acids inhibition of GAPDH leads to inhibition of glycolysis. 2. Inhibition of glycolysis leads to reduced ATP levels and repressed pyruvate generation. 3. Reduced pyruvate leads to mitochondrial stress, ROS generation, cytotoxicity, and DNA damage.
Induction of oxidative stress	Oxidative stress	1. Haloacetic acids induce oxidative stress through multiple pathways. 2. Oxidative stress can cause mutations and damage to proteins, lipids, and DNA. 3. Mutations and damage to macromolecules activate cell-signaling pathways, induce genomic instability, and cell transformation and lead to cancer.

https://ntp.niehs.nih.gov/ntp/about_ntp/monopeerrvw/2017/july/haafinalmonograph_508.pdf

Limitations of MOA/AOP Approach

- Biology is not linear – influenced by feedback mechanisms, repair, background, susceptibilities…Network of systems
- Multiple ways to arrive at same conclusion – Does not fit with Causal Pie concept
- Limited by the current understanding of the disease process (recognized by Sir Bradford Hill, who noted that “what is biologically plausible depends upon the biological knowledge of the day”)
- Key events are supposed to be quantifiable but in reality they may be impossible to measure

Limitations of MOA/AOP Approach (continued)

- MOA/AOP may be incomplete or wrong [e.g. DEHP – Rusyn and Corton (2012)]
- Focus on 'favorite' mechanism may introduce bias, especially on committees and public databases
- How many 'validated' AOPs needed for 100K chemicals producing 1000s of adverse outcomes in different ways?

Key characteristics don't require risk assessor to guess the mechanism

- Mechanistic hypotheses in science are beneficial because if you test it and are wrong then you modify the hypothesis and get closer to the truth

- Mechanistic hypotheses in risk assessment are problematic because if you are wrong you may have made a bad risk decision that cannot easily be changed and may have caused medical or economic harm

Using 21st Century Science to Improve Risk-Related Evaluations - Comments

- The KC "approach avoids a narrow focus on specific pathways and hypotheses and provides for a broad, holistic consideration of the mechanistic evidence." (P.144)
- "The committee notes that key characteristics for other hazards, such as cardiovascular and reproductive toxicity, could be developed as a guide for evaluating the relationship between perturbations observed in assays, their potential to pose a hazard, and their contribution to risk." (p.141)
- Through a project funded by OEHHA (Cal EPA), KCs for reproductive toxicants and endocrine disruptors have been developed

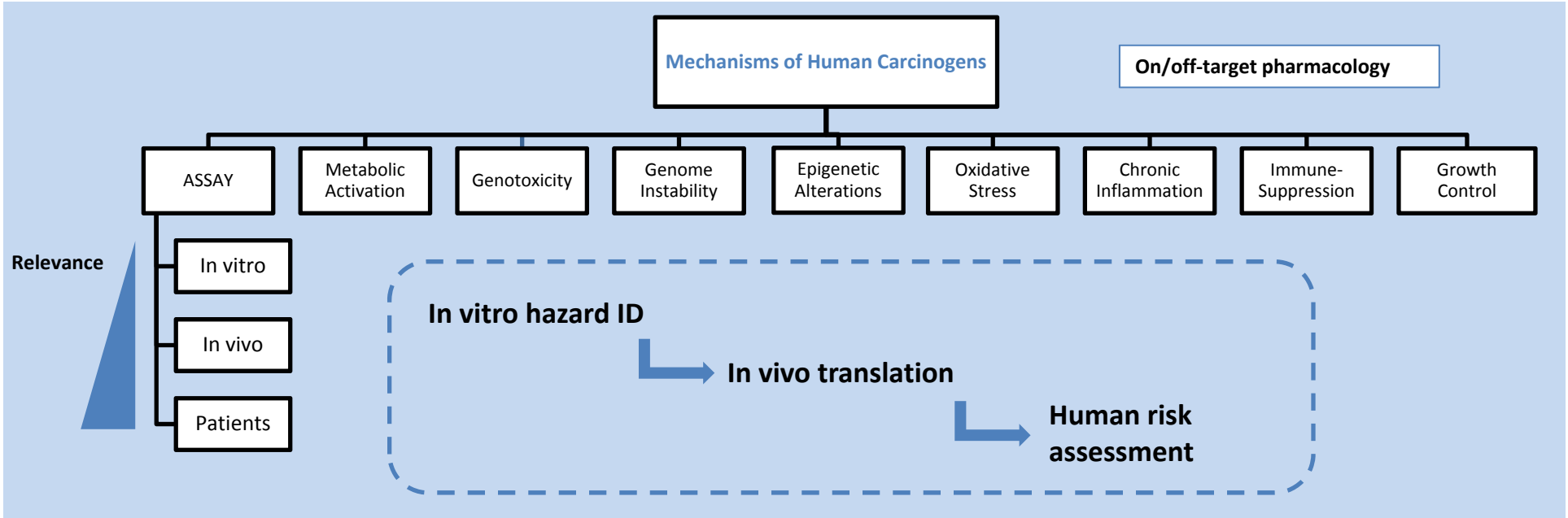
Working Group on KCs of Endocrine Disruptors and Reproductive Toxicants

Berkeley CA, March 7-8, 2018

What Next for the Key Characteristics?

- Refinement of definitions and listing of all assays for each characteristic
- Development of HT assays specific for each characteristic – A CarciCAST – Testing of new drugs and chemicals (see Fielden et al. 2018)
- Key characteristics of other endpoints – cardiovascular toxicity; developmental toxicity etc.

Use of KC's for assessment of therapeutics



Hypothesis: Evaluating the Key Characteristics will provide a more comprehensive and predictive assessment of human cancer risk than evaluating tumors in rodent bioassays

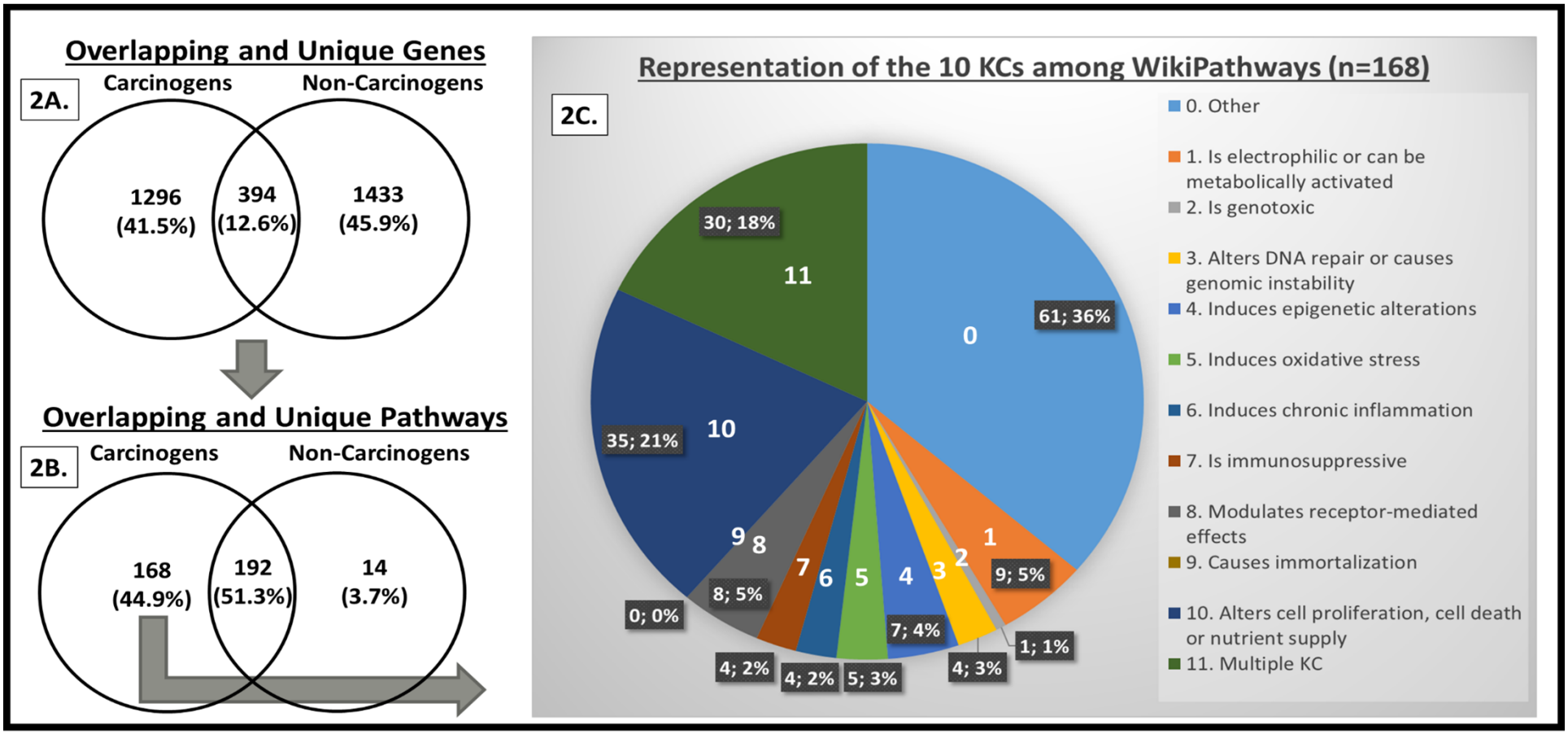
Growth control: proliferation, apoptosis, immortalization, metabolism

Adapted from Fielden *et al* ***Trends Pharmacol Sci. 2018***

Question for the Future

Can we predict that a chemical possesses multiple key characteristics using HTS/ toxicogenomic data and prioritize it for further evaluation as a possible/probable human carcinogen?

Using the Key Characteristics in a Data Science Approach to Prioritize Chemicals for Hazard Identification – Linda Rieswijk et al



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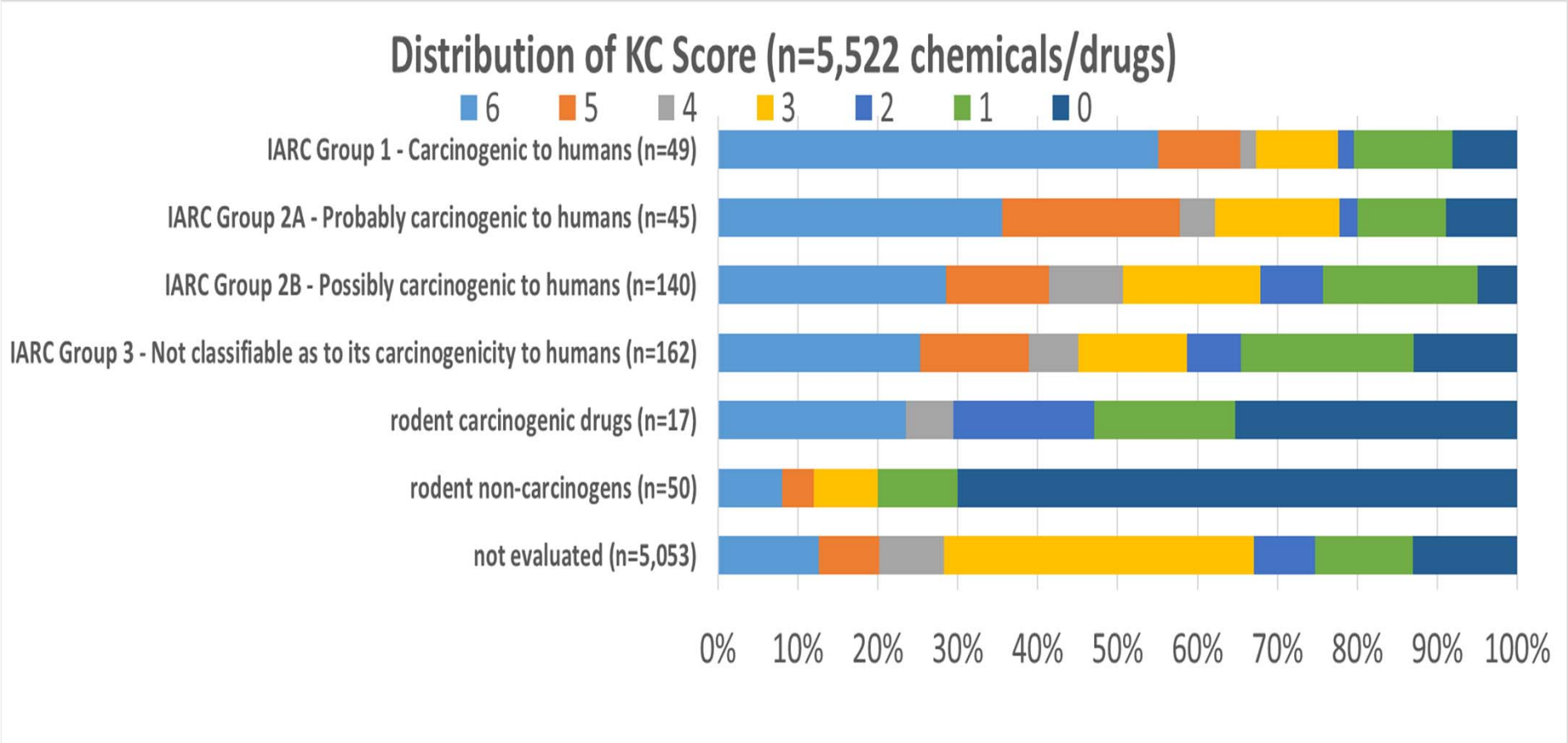


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Thank you for listening!