SOT CTSS and CSS Joint Webinar
"Integrating Computational Tools into Carcinogenicity Assessments - Part II"

In Silico Approaches In Carcinogenicity Hazard Assessment: Current Status and Future Needs.

February 15, 2022

Raymond Tice, Ph.D.
RTice Consulting
Hillsborough, NC
rrtice47@gmail.com

Topics

- The *In Silico* Protocol Consortium
- The *In Silico* Carcinogenicity Protocol Working Group
- The *In Silico* Carcinogenicity Case Studies Working Group
- The ICH S1B (addendum) Working Group
- Evaluating Confidence in Toxicity Assessments

In silico toxicology protocols

Regulatory Toxicology and Pharmacology 96 (2018) 1–17

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

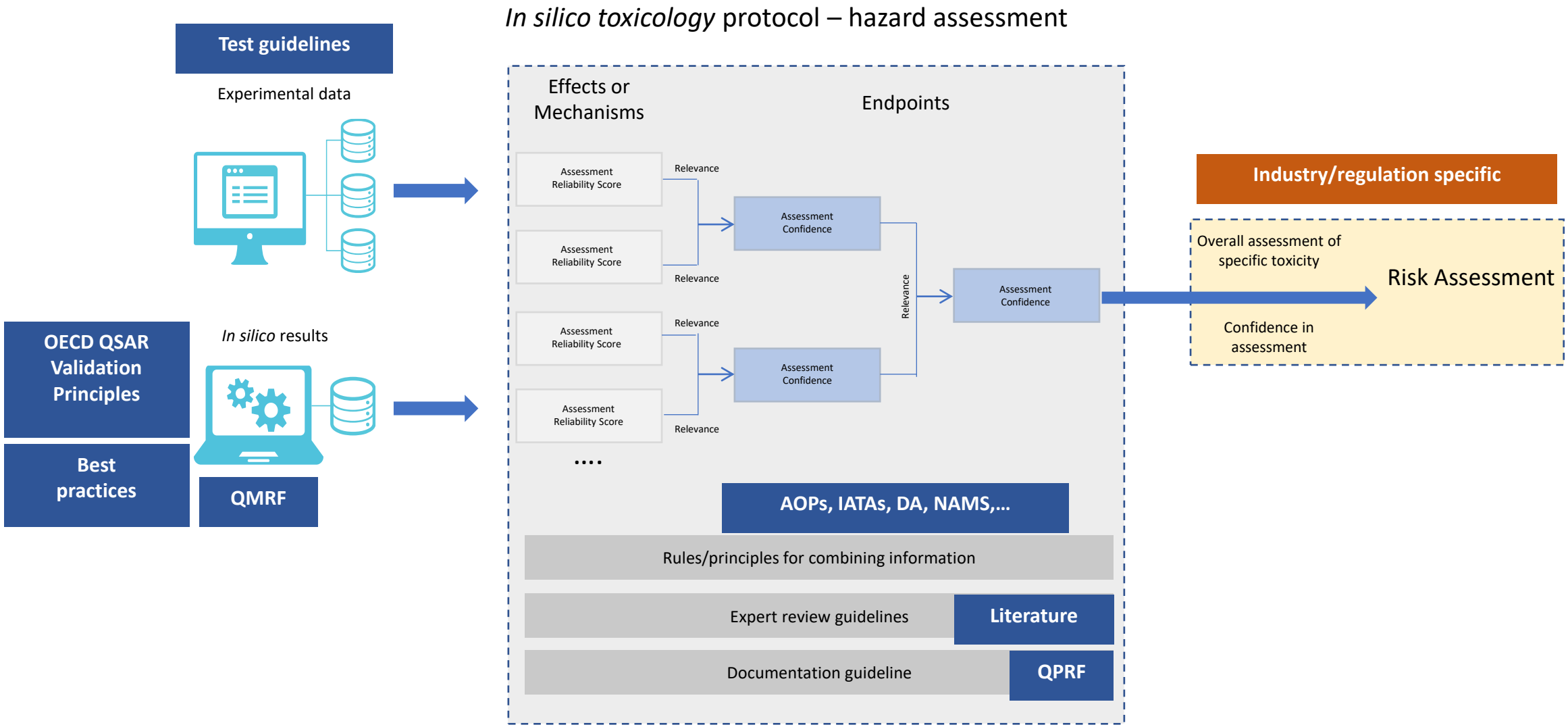
In silico toxicology protocols

Glenn J. Myatt[a,*], Ernst Ahlberg[b], Yumi Akahori[c], David Allen[d], Alexander Amberg[e], Lennart T. Anger[e], Aynur Aptula[f], Scott Auerbach[g], Lisa Beilke[h], Phillip Bellion[i], Romualdo Benigni[j], Joel Bercu[k], Ewan D. Booth[l], Dave Bower[a], Alessandro Brigo[m], Natalie Burden[n], Zoryana Cammerer[o], Mark T.D. Cronin[p], Kevin P. Cross[a], Laura Custer[q], Magdalena Dettwiler[r], Krista Dobo[s], Kevin A. Ford[t], Marie C. Fortin[u], Samantha E. Gad-McDonald[v], Nichola Gellatly[n], Véronique Gervais[w], Kyle P. Glover[x], Susanne Glowienke[y], Jacky Van Gompel[z], Steve Gutsell[f], Barry Hardy[aa], James S. Harvey[bb], Jedd Hillegass[q], Masamitsu Honma[cc], Jui-Hua Hsieh[dd], Chia-Wen Hsu[ee], Kathy Hughes[ff], Candice Johnson[a], Robert Jolly[gg], David Jones[hh], Ray Kemper[ii], Michelle O. Kenyon[s], Marlene T. Kim[ee], Naomi L. Kruhlak[ee], Sunil A. Kulkarni[ff], Klaus Kümmerer[jj], Penny Leavitt[q], Bernhard Majer[kk], Scott Masten[g], Scott Miller[a], Janet Moser[ll,mm], Moiz Mumtaz[nn], Wolfgang Muster[m], Louise Neilson[oo], Tudor I. Oprea[pp], Grace Patlewicz[qq], Alexandre Paulino[rr], Elena Lo Piparo[ss], Mark Powley[ee], Donald P. Quigley[a], M. Vijayaraj Reddy[tt], Andrea-Nicole Richarz[uu], Patricia Ruiz[nn], Benoit Schilter[ss], Rositsa Serafimova[vv], Wendy Simpson[f], Lidiya Stavitskaya[ee], Reinhard Stidl[kk], Diana Suarez-Rodriguez[f], David T. Szabo[ww], Andrew Teasdale[xx], Alejandra Trejo-Martin[k], Jean-Pierre Valentin[yy], Anna Vuorinen[j], Brian A. Wall[zz], Pete Watts[aaa], Angela T. White[bb], Joerg Wichard[bbb], Kristine L. Witt[g], Adam Woolley[ccc], David Woolley[ccc], Craig Zwickl[ddd], Catrin Hasselgren[a]

[a] Leadscope, Inc., 1393 Dublin Rd, Columbus, OH 43215, USA
[b] Predictive Compound ADME & Safety, Drug Safety & Metabolism, AstraZeneca IMED Biotech Unit, Mölndal, Sweden
[c] Chemicals Evaluation and Research Institute, 1-4-25 Kouraku, Bunkyo-ku, Tokyo 112-0004 Japan
[d] Integrated Laboratory Systems, Inc., Research Triangle Park, NC, USA
[e] Sanofi, R&D Preclinical Safety Frankfurt, Industriepark Hoechst, D-65926 Frankfurt am Main, Germany
[f] Unilever, Safety and Environmental Assurance Centre, Colworth, Beds, UK
[g] The National Institute of Environmental Health Sciences, Division of the National Toxicology Program, Research Triangle Park, NC 27709, USA
[h] Toxicology Solutions Inc., San Diego, CA, USA
[i] DSM Nutritional Products, Kaiseraugst, Switzerland
[j] Alpha-PreTox, via G.Pascoli 1, 00184 Roma, Italy
[k] Gilead Sciences, 333 Lakeside Drive, Foster City, CA, USA
[l] Syngenta, Product Safety Department, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK
[mm] Roche Pharmaceutical Research & Early Development, Pharmaceutical Sciences, Roche Innovation Center Basel, Switzerland
[nn] National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), Gibbs Building, 215 Euston Road, London NW1 2BE, UK

- Equivalent to *in vivo* or *in vitro* test guidelines
- Developed through an international cross-industry consortium comprised of over 70 organizations including regulators, government agencies, industry (pharmaceutical, agrochemicals, industrial chemicals, consumer products), academics, model developers, consultants, NGOs
- Individual working group established per major toxicological endpoint
- These protocols help ensure any assessment is performed in a transparent, accepted, consistent, documented, and repeatable manner
- They incorporate:
  1. Best practices in computational toxicology
  2. The current science in assessing toxicity weight of the evidence (as encoded in AOPs, IATAs, and so on)

In silico toxicology protocol framework - leveraging existing work



From G. Myatt (Instem)

Computational Toxicology

4.4
CiteScore

The in silico toxicology protocols initiative

Edited by Glenn Myatt, Kevin Cross, Candice Johnson
Last update 23 September 2021

In silico toxicology (IST) encompasses the use of computational models, based on different methodologies, to make predictions of the toxicity of a chemical. IST is being used to support an increasing number of applications for both regulatory purposes as well as to support industrial use cases. This increased usage is driven by several factors, including the desire to reduce animal testing and the need to increase the efficiency and overall safety of bringing new chemical products to market. The use of in silico assessment has in turn driven the development of IST best practices to ensure their usage is both transparent and defendable. Computational frameworks that incorporate such in silico practices will have a critical role in the expanding area of IST.

Research article ○ Abstract only

In silico approaches in organ toxicity hazard assessment: Current status and future needs in predicting liver toxicity

Arianna Bassan, Vinicius M. Alves, Alexander Amberg, Lennart T. Anger, ... Glenn J. Myatt

November 2021

Article 100187

Purchase PDF | Article preview

Research article ○ Abstract only

In silico approaches in organ toxicity hazard assessment: Current status and future needs for predicting heart, kidney and lung toxicities

Arianna Bassan, Vinicius M. Alves, Alexander Amberg, Lennart T. Anger, ... Glenn J. Myatt

November 2021

Article 100188

Purchase PDF | Article preview

Research article ○ Abstract only

In silico approaches in carcinogenicity hazard assessment: Current status and future needs

Raymond R. Tice, Arianna Bassan, Alexander Amberg, Lennart T. Anger, ... Glenn J. Myatt

November 2021

Article 100191

Purchase PDF | Article preview

Research article ● *Open access*

Implementation of *in silico* toxicology protocols within a visual and interactive hazard assessment platform

Glenn J. Myatt, Arianna Bassan, Dave Bower, Candice Johnson, ... Kevin P. Cross

February 2022

Article 100201

Download PDF | Article preview

Research article ○ Abstract only

Evaluating confidence in toxicity assessments based on experimental data and *in silico* predictions

Candice Johnson, Lennart T. Anger, Romualdo Benigni, David Bower, ... Glenn J. Myatt

February 2022

Article 100204

Purchase PDF | Article preview

Research article ○ Abstract only

Increasing the acceptance of *in silico* toxicology through development of protocols and position paper

Glenn J. Myatt, Arianna Bassan, Dave Bower, Kevin M. Crofton, ... Candice Johnson

February 2022

Article 100209

In Silico Carcinogenicity Protocol Working Group Position Paper

Tice RR, Bassan A, Amberg A, Anger LT, Beal MA, Bellion P, Benigni R, Birmingham J, Brigo A, Bringezu F, Ceriani L, Crooks I, Cross K, Elespuru R, Faulkner DM, Fortin MC, Fowler P, Frericks M, Gerets HHJ, Jahnke GD, Jones DR, Kruhlak NL, Lo Piparo E, Lopez-Belmonte J, Luniwal A, Luu A, Madia F, Manganelli S, Manickam B, Mestres J, Mihalchik-Burhans AL, Neilson L, Pandiri A, Pavan M, Rider CV, Rooney JP, Trejo-Martin A, Watanabe-Sailor KH, White AT, Woolley D, Myatt GJ.

In Silico Approaches In Carcinogenicity Hazard Assessment: Current Status and Future Needs

Computational Tox 20, 100191, 2021. https://doi.org/10.1016/j.comtox.2021.100191.

Key Characteristics of Carcinogens*

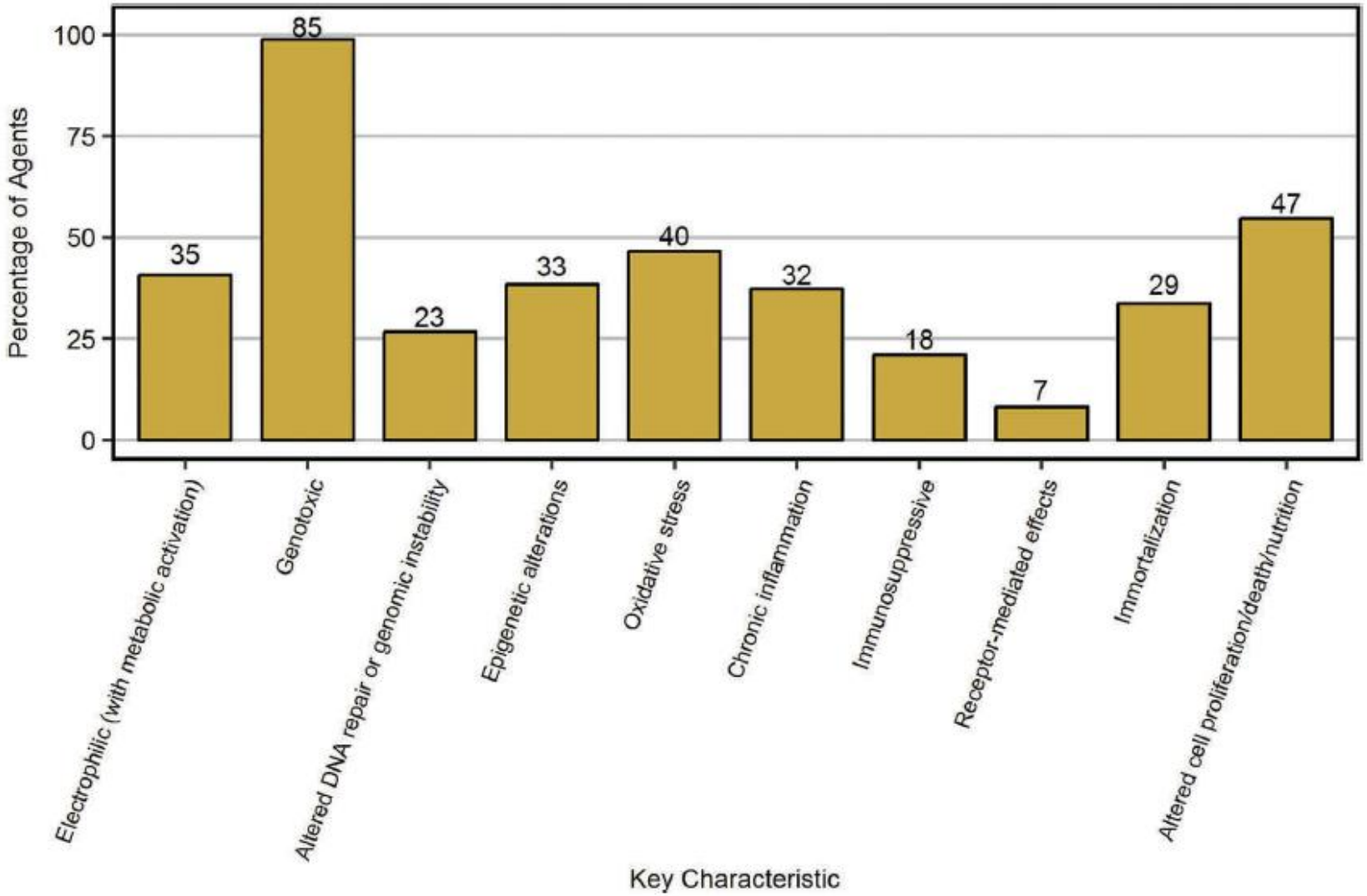
1. Is electrophilic or can be metabolically activated
2. Is genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces epigenetic alterations
5. Induces oxidative stress
6. Induces chronic inflammation
7. Is immunosuppressive
8. Modulates receptor-mediated effects
9. Causes immortalization
10. Alters cell proliferation, cell death, or nutrient supply

*Adapted from Smith et al. Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis. Environ. Health Perspect. 2016, 124: 713–721.

In Silico Carcinogenicity Position Paper Goals

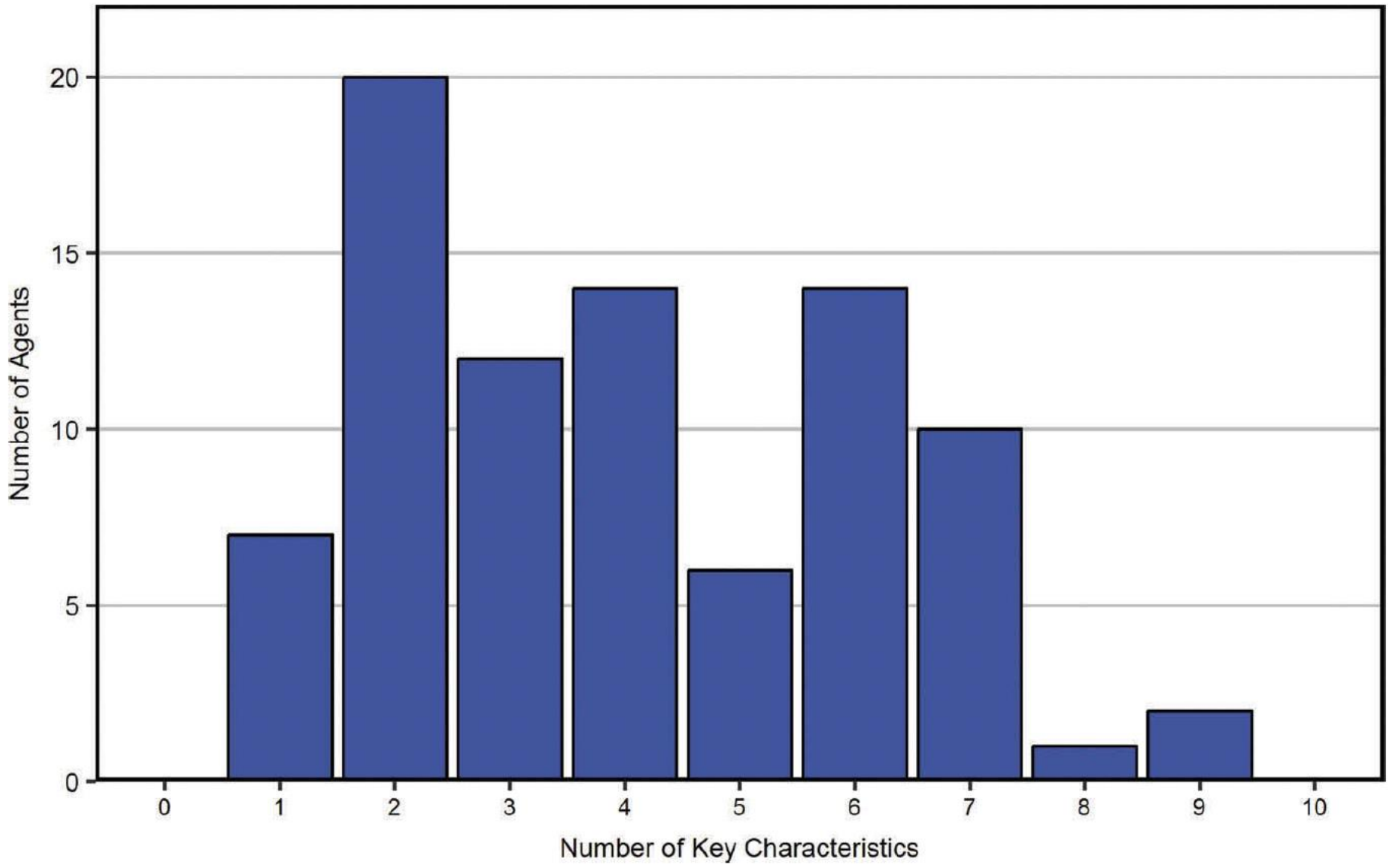
- define the key characteristic/mechanism and how it contributes to carcinogenicity
- define relevant processes/endpoints that (could) lead to the key characteristic/mechanism
- identify *in vitro* and/or *in vivo* experimental approaches for detecting chemicals that induce the key characteristic/mechanism, pointing out emerging experimental approaches
- identify computational methods (statistical models, expert alerts, read-across) that are available or are not available to identify chemicals that potentially induce a key characteristic/mechanism
- identify experimental approaches that are needed to generate relevant data and/or identify data gaps that are needed to develop computational methods to analyze such data

Key Characteristics of IARC 86 Group 1 Agents*

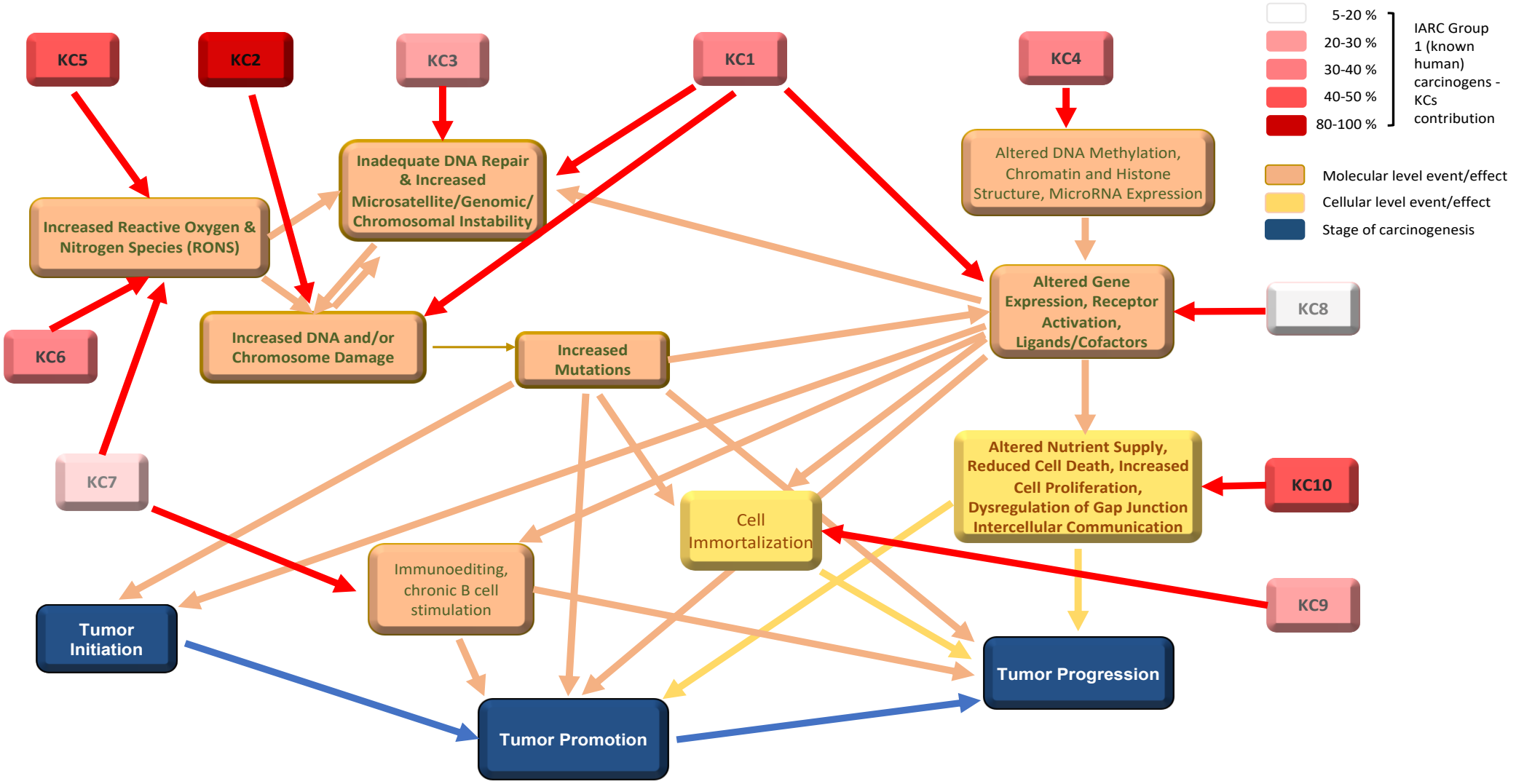


*Krewski et al. (2019) J Toxicol Environ Hlth, Part B, 22:7-8, 244-263, DOI: 10.1080/10937404.2019.1643536

Number of IARC Group 1 Agents Demonstrating One or More Key Characteristics*



*Krewski et al. (2019) J Toxicol Environ Hlth, Part B, 22:7-8, 244-263, DOI: 10.1080/10937404.2019.1643536



Interactions among the 10 KCs and with the Three Stages of Carcinogenesis

KC1 = is electrophilic, KC2 = is genotoxic, KC3 = alters DNA repair or causes genomic instability, KC4 = induces epigenetic alterations, KC5 = induces oxidative stress, KC6 = induces chronic inflammation, KC7 = is immunosuppressive, KC8 = modulates receptor-mediated effects, KC9 = causes immortalization, KC10 = alters cell proliferation, cell death, or nutrient supply. Supporting information for the different arrows can be found in the text or are considered by the working group to be biologically plausible. KCs are color coded according to the association between KCs and 86 chemicals carcinogenic to humans (International Agency for Research on Cancer Group 1 Agents) as determined by Krewski et al. (2019).

Relationships between the different KCs, their contribution to one or more stages of carcinogenesis, and availability of global *in silico* methods and whether used for making regulatory decisions related to cancer

Key Characteristic (KC)	First level, common molecular or cellular level event/effect among KCs	In Silico Methods	
		Availability	Used for making cancer-related regulatory decisions
KC1: Is Electrophilic or Can Be Metabolically Activated	KC2, KC3, KC8	Well-established for adduct formation, but otherwise limited	Yes, but limited to DNA reactivity
KC2: Is Genotoxic	KC1	Well-established	Yes
KC3: Alters DNA Repair Or Causes Genomic Instability	KC1	None	No
KC4: Induces Epigenetic Alterations		Limited	No
KC5: Induces Oxidative Stress	KC6, KC7	Not for cancer but for assay specific endpoints	No
KC6: Induces Chronic Inflammation	KC5, KC7	Not for cancer but could be developed for assay specific endpoints	No
KC7: Is Immunosuppressive	KC5, KC6	None	No
KC8: Modulates Receptor-Mediated Effects	KC1	Yes, for multiple nuclear receptors	No
KC9: Causes Immortalization		Only for *in vitro* Syrian hamster embryo cell transformation	No
KC10: Alters cell proliferation, cell death, or nutrient supply		None	No

First level refers to arrows from two or more KCs that point to the same first molecular or cellular level event/effect. KCs 1-8 contribute to all three stages of carcinogenesis (initiation, promotion, progression), while KCs 9 and 10 contribute to promotion and progression

General Conclusions

- With the exception of KC2 – is genotoxic - there is a lack of global computational methods as they relate to carcinogenicity.
- For some KCs, assays exist that could be used to provide robust data for developing computational methods; not yet true for others.
- Given that many of the KCs interact with each other, it is unclear how much information is sufficient and how the data should be combined to generate an overall assessment along with an associated level of confidence.

In Silico Carcinogenicity Case Studies Working Group

- Goal is to develop a series of **case studies** to support the development of new approaches and protocols, using information from:
  - target safety assessments
  - *in silico* approaches
  - *in vitro* data
  - *in vivo* data
- Where such approaches are fit-for-purpose and based on a human-oriented quantitative assessment
  - To improve the speed and accuracy of carcinogenicity assessment, while avoiding animal experiments as much as practically possible,
  - Takes into consideration the risk of false negatives
  - Include if possible an assessment of exposure and genetic diversity
- These approaches may be used for:
  - Regulatory use cases
  - Pharmaceuticals/plant protection products industrial use cases
  - Provide sufficient information to waive a 2-year rodent bioassay

Chemicals Selected for Case Studies

- Nongenotoxic and carcinogenic – pregabalin, an anti-epileptic drug that induces tumors in mice only (Doug Keller; 21stcenturytox@gmail.com)
- Nongenotoxic and noncarcinogenic – acetaminophen, extensively evaluated (Frank Bringezu; frank.bringezu@merckgroup.com)
- Genotoxic and carcinogenic – lasiocarpine, a pyrrolizidine alkaloid classified by IARC as being possibly carcinogenic to humans (category 2B) (Elena Lo Piparo; elena.lopiparo@rdls.nestle.com)
- Genotoxic and noncarcinogenic - quinacrine, a mutagenic drug widely used to prevent malaria

ICH S1B WORKING GROUP

(Arianna Bassan; arianna.bassan@innovatune.com)

The recently published addendum of the ICH S1B guideline introduces a weight-of-evidence (WoE) approach to assesses the human carcinogenic potential of small molecule pharmaceuticals **and to determine whether a 2-year rat carcinogenicity study would add value**

Application of this integrative approach would reduce the use of animals in accordance with the **3Rs** principles, and shift resources to focus onto generating more scientific **mechanism-based** carcinogenicity assessments

To support this guideline, a working group is being established to develop a protocol for performing such an assessment in a transparent, consistent, and defendable manner

ICH
harmonisation for better health

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

ADDENDUM TO THE GUIDELINE ON TESTING FOR CARCINOGENICITY OF PHARMACEUTICALS S1B(R1)

Draft version

Endorsed on 10 May 2021

Currently under public consultation

ICH S1B Addendum – Integrative WoE Assessment

An **integrative WoE assessment** approach may support a conclusion that the test compound is either:

1. **Likely to be carcinogenic in humans** such that the product would be labeled accordingly and any 2-year rat carcinogenicity studies would not add value.
2. **Likely not to be carcinogenic in humans** such that a 2-year rat study would not add value (may also not be carcinogenic in rats, or may likely be carcinogenic in rats but through well recognized mechanisms known to be human irrelevant).
3. **Uncertain** with respect to the carcinogenic potential for humans, and a 2-year rat carcinogenicity study is likely to add value to human risk assessment.

Objectives of the S1B Working Group

1. Map the KCs of carcinogens to the ICH S1B WoE factors
2. Develop a pragmatic hazard assessment framework (based on the principles of the *in silico* toxicology protocols) to support case studies
3. Develop an implementation of the protocol to support case studies
4. Consider how available protocols would be integrated
5. Propose weight of evidence rules/principles for combining information and deriving a confidence score

Computational Toxicology
Volume 21, February 2022, 100204

Evaluating confidence in toxicity assessments based on experimental data and *in silico* predictions

Candice Johnson [a], Lennart T. Anger [b], Romualdo Benigni [c], David Bower [a], Frank Bringezu [d], Kevin M. Crofton [e], Mark T.D. Cronin [f], Kevin P. Cross [a], Magdalena Dettwiler [g], Markus Frericks [h], Fjodor Melnikov [b], Scott Miller [a], David W. Roberts [f], Diana Suarez-Rodrigez [i], Alessandra Roncaglioni [j], Elena Lo Piparo [k], Raymond R. Tice [l], Craig Zwickl [m], Glenn J. Myatt [a]

Show more

Share Cite

https://doi.org/10.1016/j.comtox.2021.100204

Reliability Scores That Consider *In Silico* Results*

Reliability Score	Klimisch Score**	Description	Summary
RS1	1	Data reliable without restriction	Well documented and accepted study or data from the literature; Performed according to valid and/or accepted test guidelines (e.g., OECD); Preferably performed according to good laboratory practices (GLP)
RS2	2	Data with restriction	Well documented and sufficient; Primarily not performed according to GLP; Partially complies with test guideline
RS3	-	Exert review	Read-across; Expert review of *in silico* result(s) and/or Klimisch 3 or 4 data
RS4	-	Multiple concurring prediction results	
RS5	-	Single acceptable *in silico* result	
RS5	3	Data not reliable	Inferences between the measuring system and test substance; Test system not relevant to exposure; Method not acceptable for the endpoint
RS5	4	Data no assignable	Not sufficiently documented for an expert review; Lack of experimental details; Referenced from short abstract or secondary literature

* Adapted from Myatt et al. (2016) Regul Toxicol Pharmacol 96:1-17; ** Klimisch et al. (1997) Regul Toxicol Pharmacol 25:1-5.

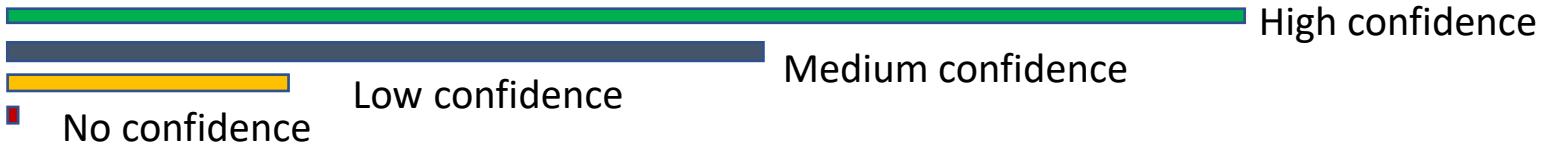
Confidence

The reliability, relevance, and coverage of information determine the level of confidence in the assessment

- A high confidence rating suggests that there is sufficient evidence that the assessment provided an accurate conclusion, and further research is unlikely to increase the confidence
- A medium confidence rating suggests that there is adequate evidence that the assessment provided an accurate conclusion, but further research might increase the confidence
- A low confidence rating suggests an accurate conclusion is lacking and further research is needed to support a robust conclusion and to improve its confidence
- A no confidence rating suggests that further research is needed in order to derive an assessment

While not appropriate for the regulatory submissions, the low confidence rating could be useful for prioritization, identification of the most relevant testing candidates and to determine data gaps.

Typically, in the case of no confidence, data are either unavailable, discordant with no supporting information or there is a lack of relevance and/or reproducibility. While decisions cannot be made in these cases, the data may be useful for discussion as seeking solutions may advance testing paradigms.



From C. Johnson

Conclusions

- It is important to determine how the scientific community could consistently and transparently review *in silico* model predictions and combine different lines of evidence to derive an overall assessment.
- In experimental systems, the concept of reliability and relevance are defined and the degree of uncertainty in an experimental system is reviewed by analyzing various experimental parameters and through a mechanistic understanding of how different chemistries interact with biological systems.
- *In silico* methods are built on computer-derived relationships between the chemical structure and biological systems, which could be explored in a manner that allows an assessment of reliability and relevance.
- Such analyses are important to better understand how much emphasis can be placed on an the prediction of an *in silico* model in a weight of evidence scenario.

Acknowledgements

- These international efforts have involved many individuals but special recognition goes to Glenn Myatt, Kevin Cross, and Candice Johnson at Leadscope/Instem; Ariana Bassan and Manuela Pavan at Innovatune Srl; Douglas Keller at 21st Century Tox, Frank Bringezu at Merck, and Elena Lo Piparo at Nestlé.
- Efforts supported in part by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number R44ES026909 to Leadscope. The content is solely the responsibility of the presenter and does not necessarily represent the official views of the National Institutes of Health.

- Individuals interested in participating in the *In Silico* Carcinogenicity Case Studies Working Group and/or the ICH S1B (addendum) Working Group should contact me at rrtice47@gmail.com.