In Silico Methods for the Prediction of Carcinogenicity

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In Silico Methods to Identify Hazard

Excellent Review: Madden JC et al (2020) *ATLA* 48: 146-172

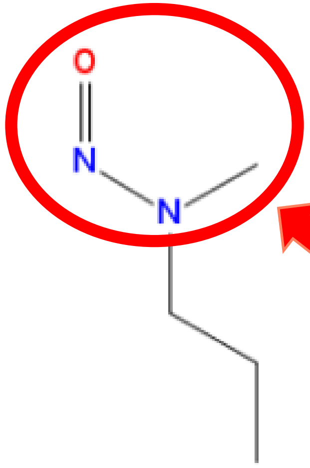
A Range of *In Silico* Approaches and Models

- Structural rules / alerts
- QSAR
- Read-Across

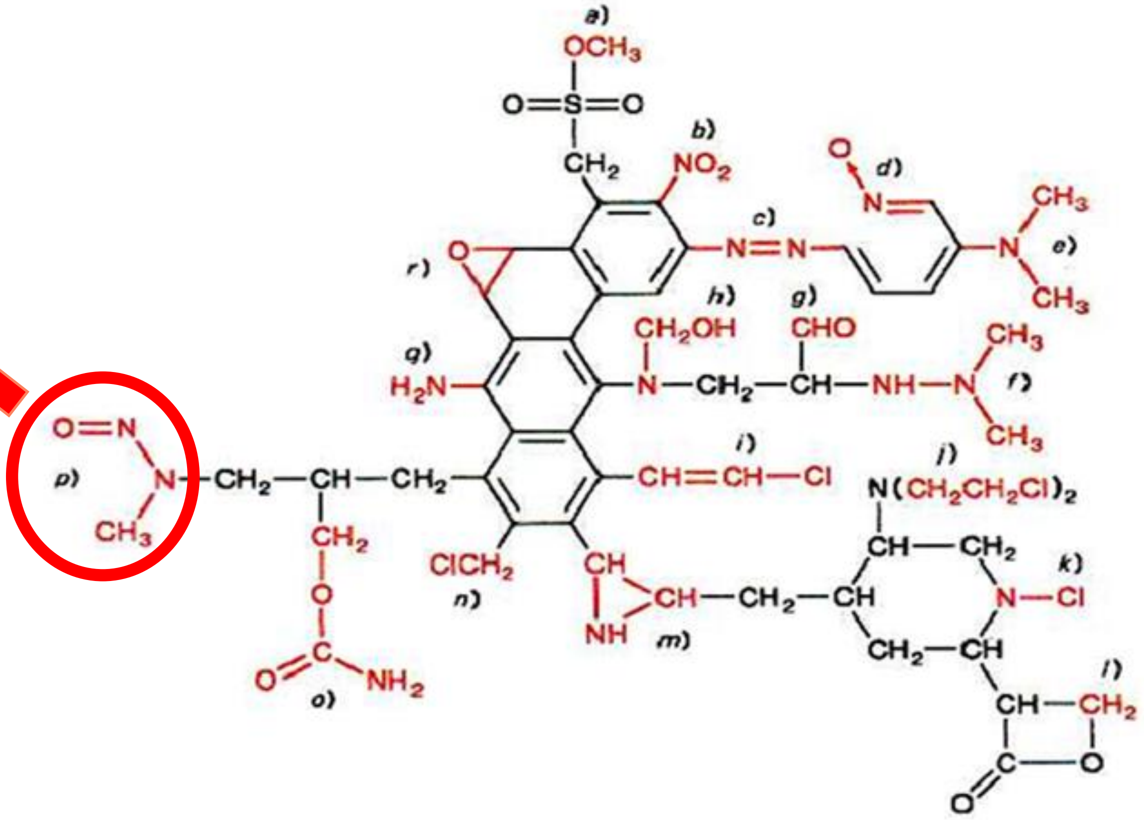
- Machine Learning / Artificial Intelligence

- Exposure
- ADME
- PBK

Structural Rules (Coded as Structural Alerts)



Potential for DNA Reactivity



Supramolecule taken from:
Ashby J, Tennant RW (1988) *Mut. Res.* 204: 17-115.

Structural Rules (Coded as Structural Alerts)

- Software (not a complete list!)
  - Toxtree
  - OncoLogic
  - OECD QSAR Toolbox
  - Derek Nexus
  - Leadscope
- Coverage and performance
  - DNA reactivity – good
  - Other genotoxicity mechanisms – moderate
  - Non-genotoxic mechanisms – poor
- Good for rapid screening, identification of highly potent compounds
- Problem of absence of alerts, metabolism etc.

Quantitative Structure-Activity Relationships (QSARs)

- QSAR seeks a relationship between carcinogenicity data and molecular properties / structural descriptors
  - Can use categorical or continuous data
  - Wide variety of techniques and approaches

Local QSAR

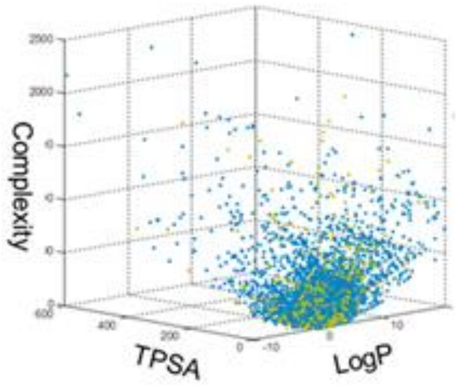
- Molecular fragment
- Chemical class
- Single mechanism
- MIE driven
- Transparent
- (Maybe) accurate

- Slow to develop
- Very slow to use
- Very restricted domain
- May not be possible for all mechanisms

Quantitative Structure-Activity Relationships (QSARs)

- QSAR seeks a relationship between carcinogenicity data and molecular properties / structural descriptors
  - Can use categorical or continuous data
  - Wide variety of techniques and approaches

Global QSAR

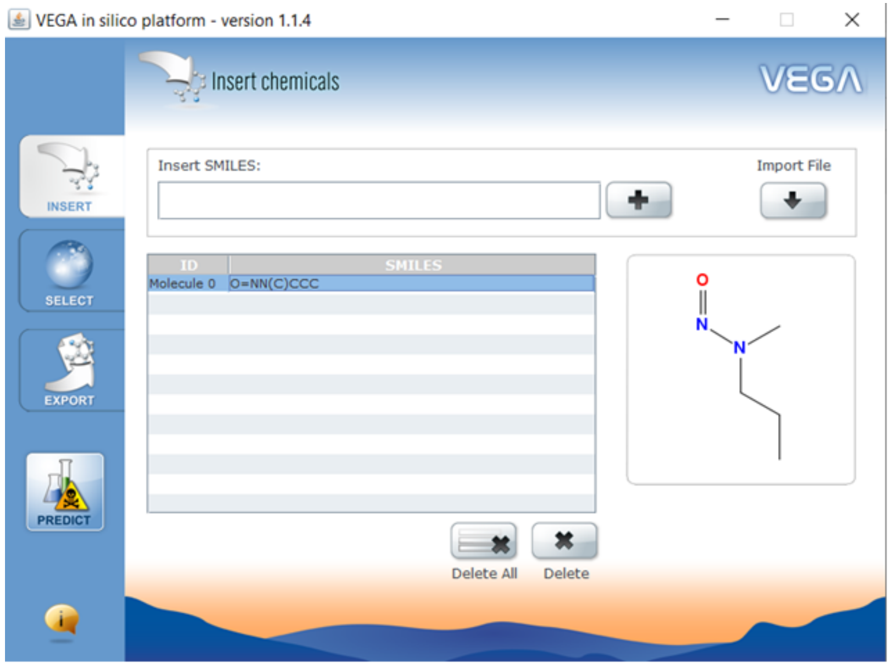


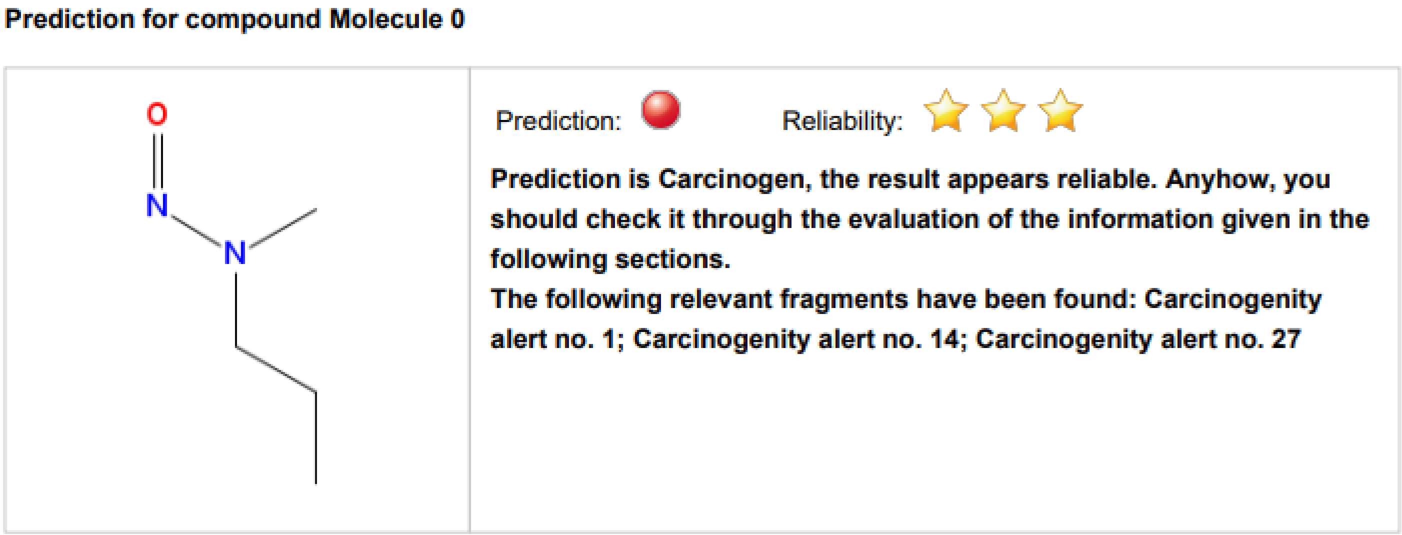
- Rapid to develop
- Rapid to use
- Broad domain
- Opportunity for Machine Learning

- Mechanisms often not considered
- (Often) black box
- Difficult to document

Quantitative Structure-Activity Relationships (QSARs)

- Software (not a complete list!)
  - Vega
  - Lazar
  - Leadscope
  - ChemTunes.ToxGPS
  - Sarah Nexus (Mutagenicity)





Performance of Models (for Mutagenicity)

Mutagenesis, 2019, 34, 3–16
doi:10.1093/mutage/gey031
Original Manuscript

Original Manuscript

Improvement of quantitative structure–activity relationship (QSAR) tools for predicting Ames mutagenicity: outcomes of the Ames/QSAR International Challenge Project

Masamitsu Honma*, Airi Kitazawa, Alex Cayley[1], Richard V. Williams[1], Chris Barber[1], Thierry Hanser[1], Roustem Saiakhov[2], Suman Chakravarti[2], Glenn J. Myatt[3], Kevin P. Cross[3], Emilio Benfenati[4], Giuseppa Raitano[4], Ovanes Mekenyan[5], Petko Petkov[5], Cecilia Bossa[6], Romualdo Benigni[6,7], Chiara Laura Battistelli[6], Alessandro Giuliani[6], Olga Tcheremenskaia[6], Christine DeMeo[8], Ulf Norinder[9,10], Hiromi Koga[11], Ciloy Jose[11], Nina Jeliazkova[12], Nikolay Kochev[12,13], Vesselina Paskaleva[13], Chihae Yang[14], Pankaj R. Daga[15], Robert D. Clark[15] and James Rathman[14,16]

Regulatory Toxicology and Pharmacology 114 (2020) 104658

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Evaluation of the applicability of existing (Q)SAR models for predicting the genotoxicity of pesticides and similarity analysis related with genotoxicity of pesticides for facilitating of grouping and read across: An EFSA funded project

Romualdo Benigni[a,*], Rositsa Serafimova[b], Juan Manuel Parra Morte[b], Chiara Laura Battistelli[c], Cecilia Bossa[c], Alessandro Giuliani[c], Elena Fioravanzo[d], Arianna Bassan[d], Mojca Fuart Gatnik[d], James Rathman[e], Chihae Yang[e], Aleksandra Mostrag-Szlichtyng[e], Oliver Sacher[e], Olga Tcheremenskaia[c]

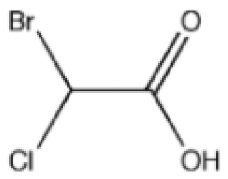
Read-Across

- Identify structural / mechanistic / metabolic analogues
- Tools such as OECD QSAR Toolbox provide a starting point
- May provide sufficient evidence to waive an animal test
- Read-across goes beyond structural similarity
- Need to consider, in detail, evidence for:
  - Mechanisms / key characteristics
  - Toxicokinetics
  - Metabolism
  - Data quality

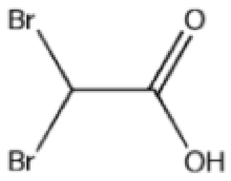
Read-Across

Source (Data Rich) Molecules

BCA



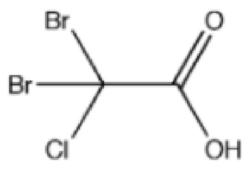
DBA



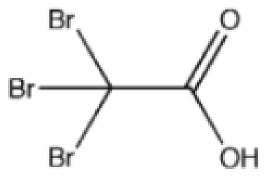
Read-across on the basis of similarity of metabolism

Target (Data Poor) Molecules

CDBA



TBA



Read-Across for Cancer Hazard Evaluation or Haloacetic Acids taken from:
Atwood ST et al (2019) *Environ. Health Persp.* 127: 125003.

The User of *In Silico* Approaches Must be Responsible!
Especially for Regulatory Applications

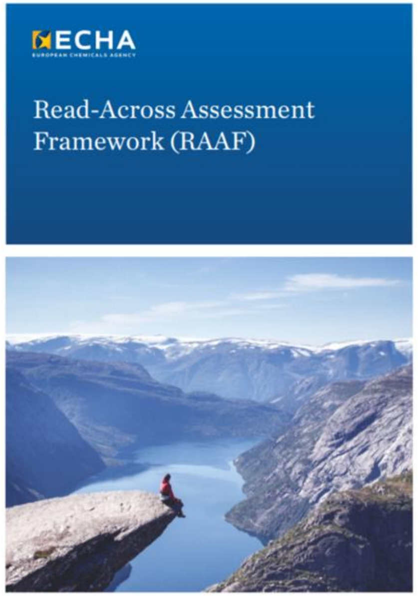
- Always ask….
  - Is the model valid?

OECD PRINCIPLES FOR THE VALIDATION, FOR REGULATORY PURPOSES, OF (QUANTITATIVE) STRUCTURE-ACTIVITY RELATIONSHIP MODELS

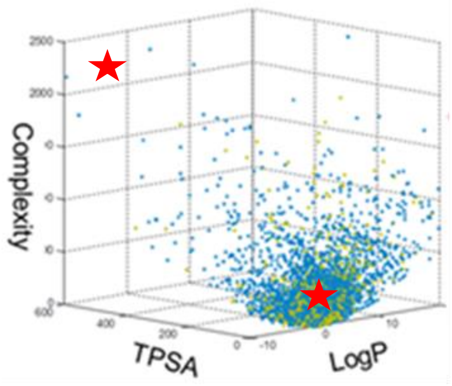
These principles were agreed by OECD member countries at the 37th Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology in November 2004. The principles are intended to be read in conjunction with the associated explanatory notes which were also agreed at the 37th Joint Meeting.

To facilitate the consideration of a (Q)SAR model for regulatory purposes, it should be associated with the following information:

1) a defined endpoint[1]
2) an unambiguous algorithm[2]
3) a defined domain of applicability[3]
4) appropriate measures of goodness-of–fit, robustness and predictivity[4]
5) a mechanistic interpretation, if possible[5]

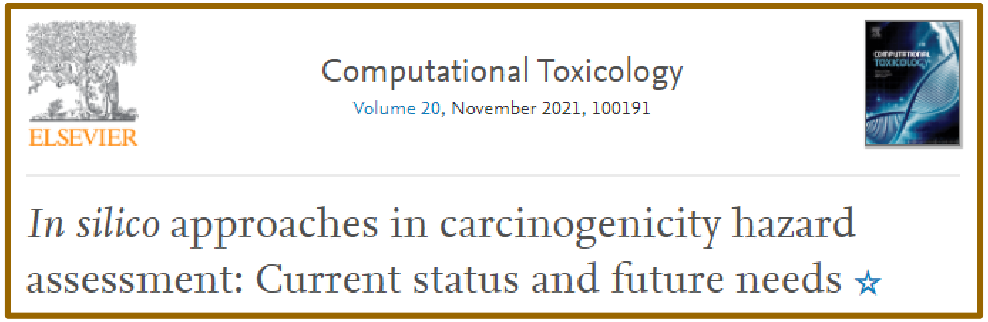


  - Is the compound in the domain of the model?



Most Important Question:
Is This Possible to Replace a 2 Year Rodent Bioassay?

Excellent Overview: Tice RR et al (2021)
Comput. Tox. 20: 100191


ELSEVIER

Computational Toxicology
Volume 20, November 2021, 100191

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

Understanding the Challenge: Complexity of the Endpoint

- Multiple mechanisms
- Dose and time dependence
- Biological / genetic / species variability
- Interpretation of data

Mechanistic Relevance: Demonstrating Causality (and Credibility)

Table 1 Key characteristics of carcinogens.

Characteristic	Examples of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

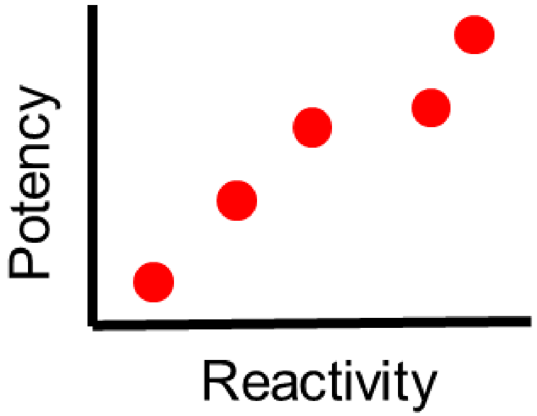
Details in: Smith MT et al (2016) *Environ. Health Persp.* 124: 713-721

Mechanistic Relevance: Demonstrating Causality (and Credibility)

- Key characteristics of carcinogens
  - Electrophilic
  - Genotoxic
  - DNA repair alteration
  - Epigenetic alteration
  - Oxidative stress
  - Chronic inflammation
  - Immunosuppressive
  - Receptor mediated
  - Immortalisation
  - Cell proliferation

***In silico* models "usually" model a factor relating to the molecular initiating event**

Local QSAR



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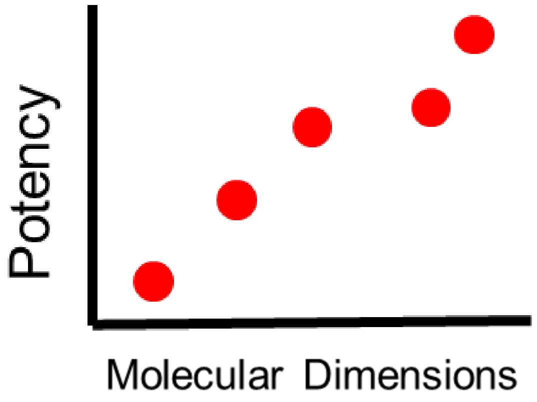

Machine Learning / Artificial Intelligence

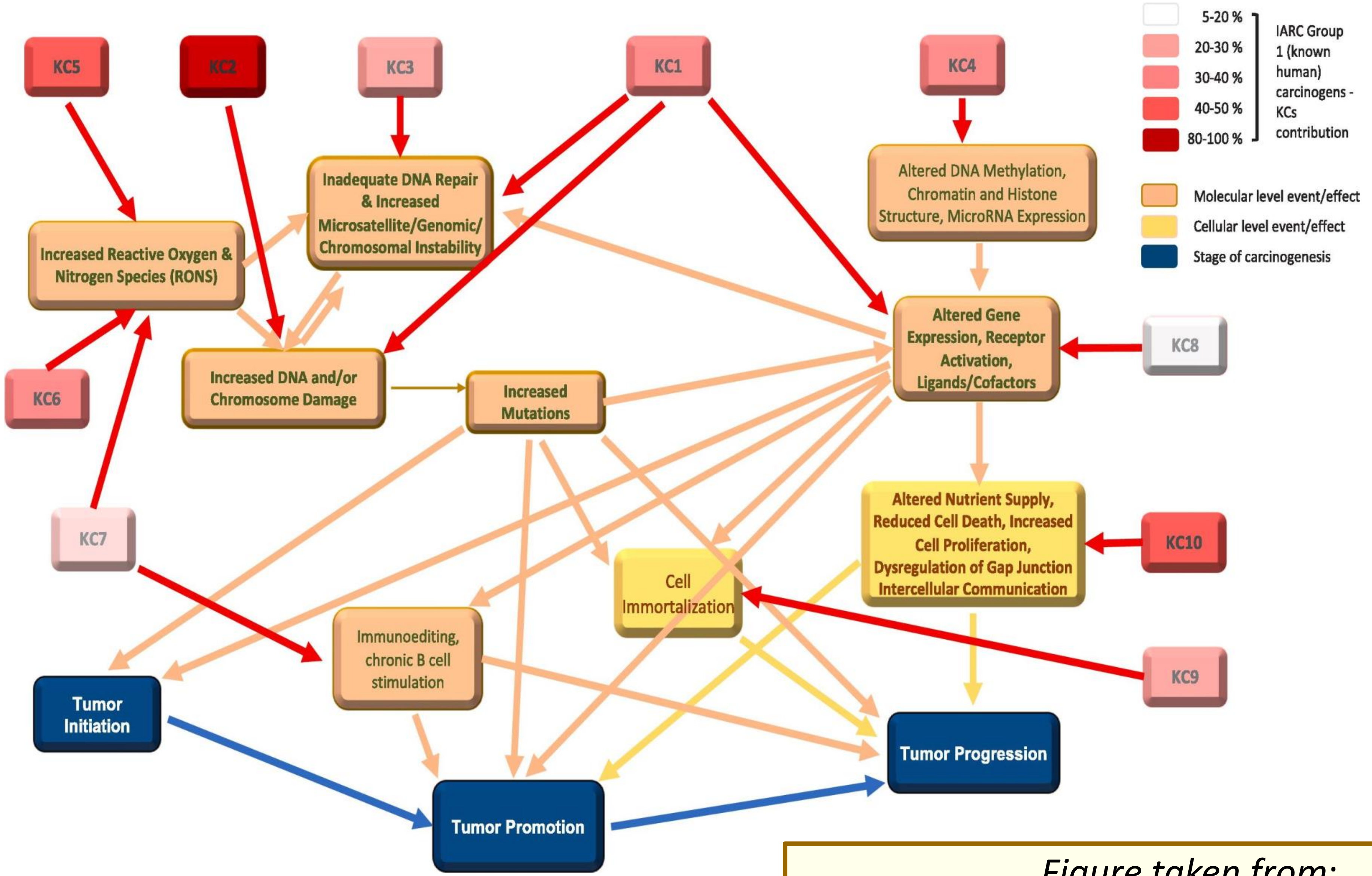

Figure taken from:
Tice RR et al (2021) *Comput. Tox.* 20: 100191.

Machine Learning / Artificial Intelligence

- Dynamic Cancer Risk Model – Potential Risk Modification Points

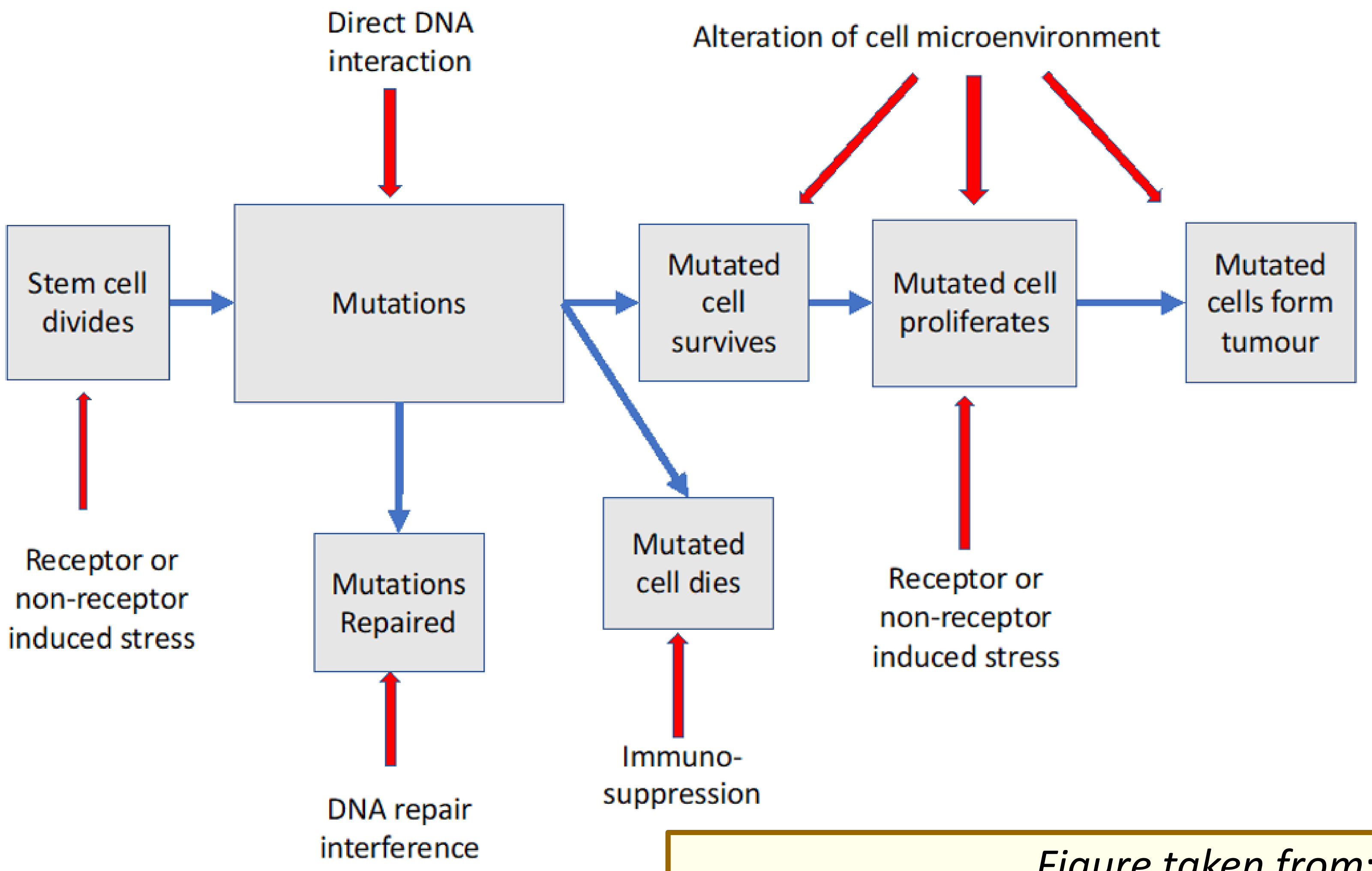

Figure taken from:
Harrison DJ and Doe JE (2021) *Toxcol. Res.* 10: 800-809.

Machine Learning / Artificial Intelligence

- IATA For Non-Genotoxic Carcinogens

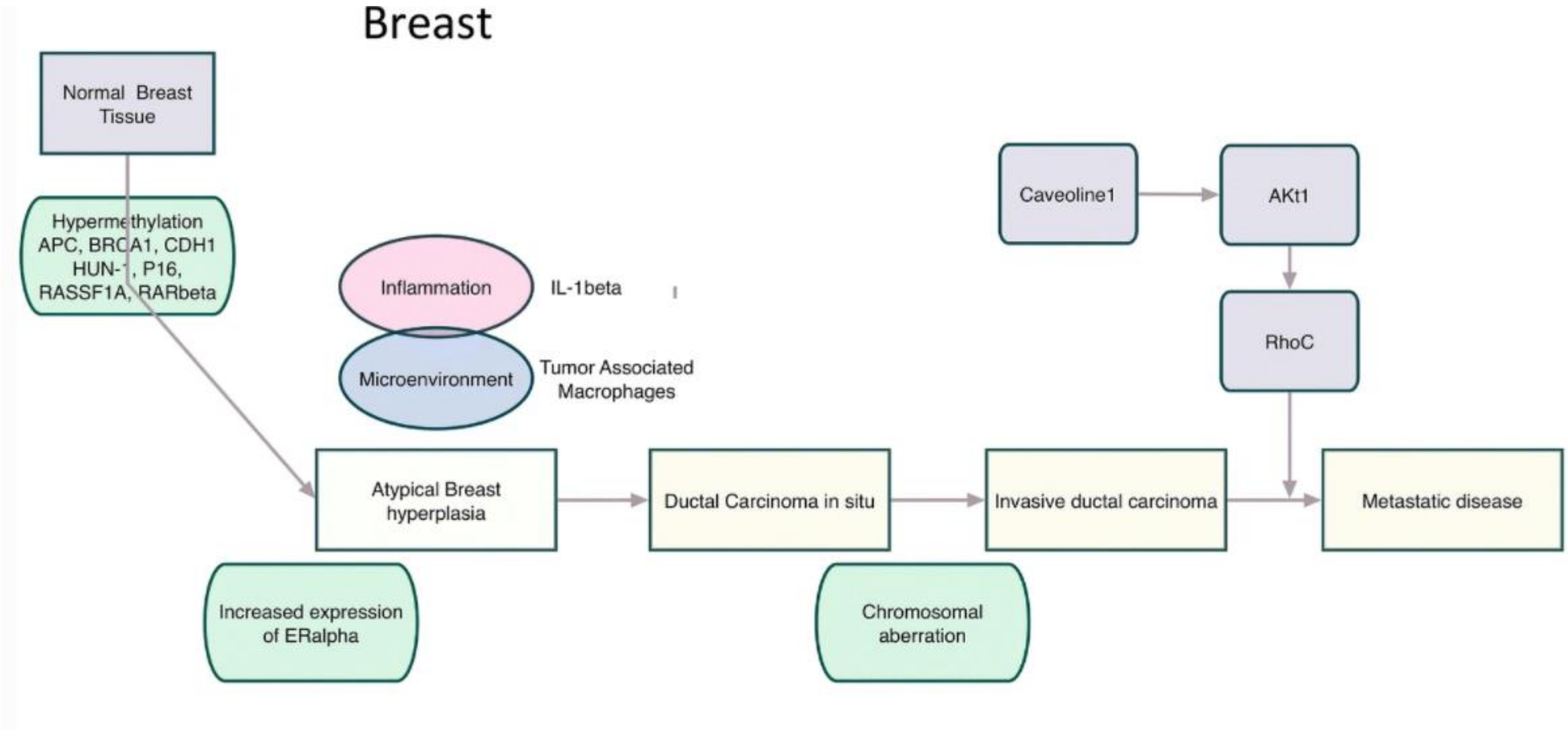

Figure taken from:
Jacobs MN et al (2020) *Arch. Toxicol.* 94: 2899–2923.

Summary

- Challenging endpoint
- Many methods and approaches
- Grounding in mechanisms / Key Characteristics
- Effective at identifying DNA reactive compounds
- Lines of evidence in an overall weight of evidence
  - Link to NAMs
- ML / AI need big data, a structure for modelling (and maybe some more thinking through!)

- Never forget the fundamentals of good modelling and that you need good data!

With Thanks To

- John Doe (Liverpool John Moores University)
- Jim Rathman, Chihae Yang (MN-AM)
- Funding from CEFIC LRi B18

- Many others!