Next Generation Assessment of Genomic Damage – "The Clean Sheet"

Path Forward for Genetic Toxicity Assessment

Kerry L. Dearfield, Ph.D.

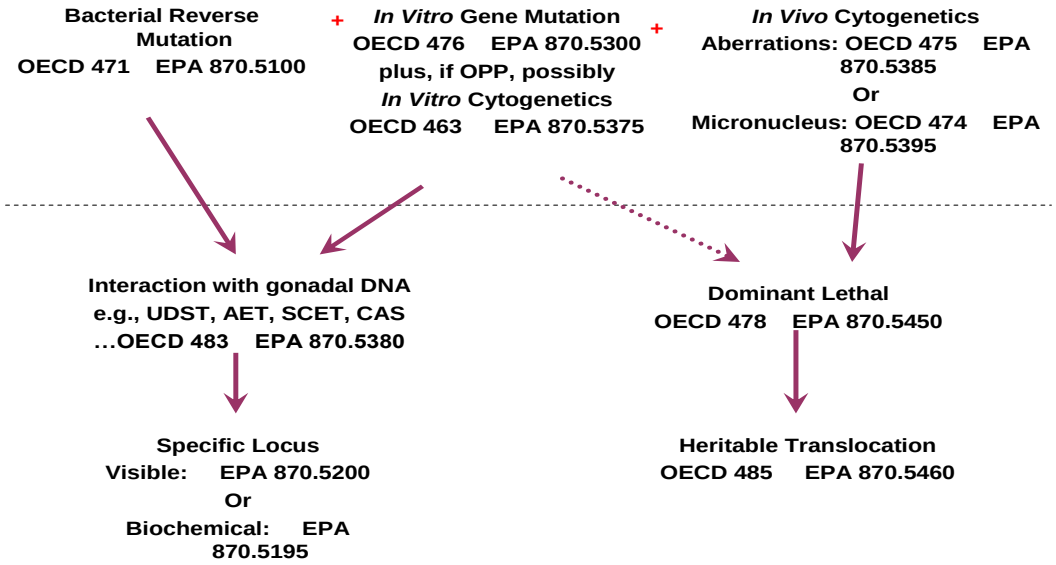
Retired. Formerly:
1) Chief Scientist
Food Safety and Inspection Service
U.S. Department of Agriculture
2) Senior Scientist for Science Policy
Environmental Protection Agency

HESI GTTC Workshop: Applied Genetic Toxicity for Regulatory Decision Making: The Road Ahead
22 March 2018

Two Messages Today

- GeneTox community moving away from hazard identification only (and just predicting cancer) to a more comprehensive assessment of potential risk to exposed humans
- Regulatory agencies now have the opportunity to embrace a more flexible, broader approach for assessing genomic damage and its implication(s) for adverse health outcomes

EPA Mutagenicity Testing Scheme for Chemicals and Pesticides



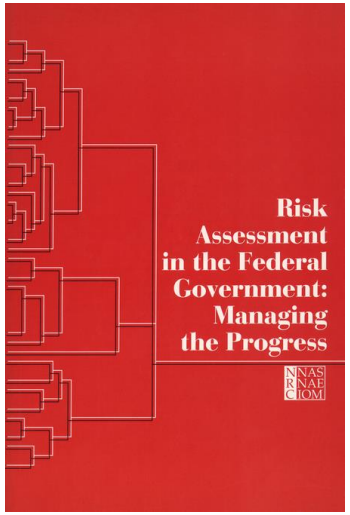
Original battery presented and discussed in Dearfield *et al.*, Mutat Res 258: 259-283, 1991

What is Risk Assessment?

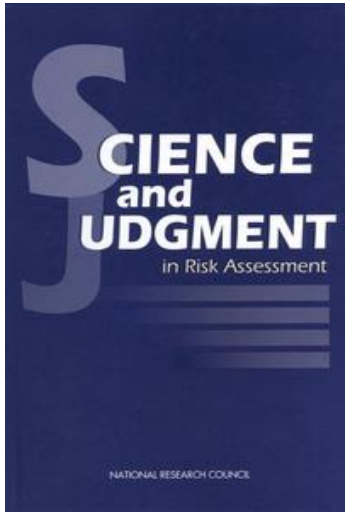
- Scientific process for estimating the probability of exposure to a hazard and the resulting public health impact (risk)
- Predicts public health benefits of changes in policies, practices, and operations
- Used to facilitate the application of science to policy (the “bridge between data and decisions”)

Risk Assessment Process

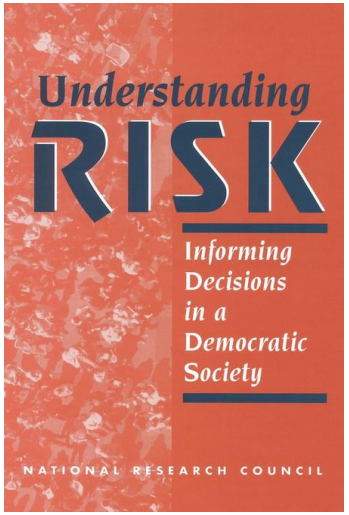
1983



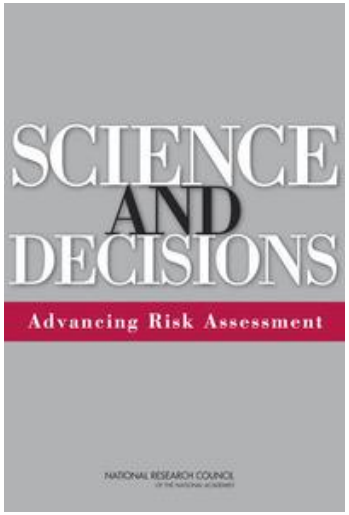
1994



1996



2009



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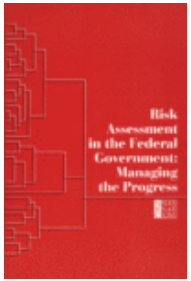
Risk Assessment Process

Codex Alimentarius process for microbial RA

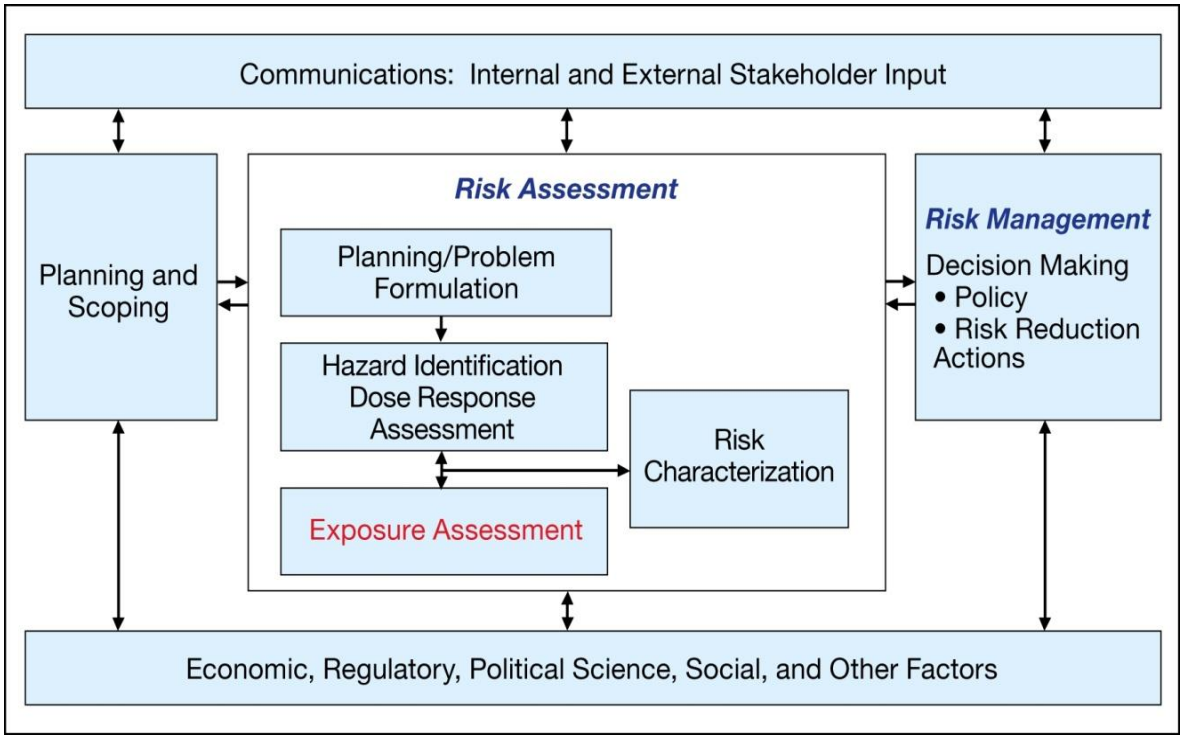
- Hazard Identification
  - In-depth literature review
- Hazard Characterization
  - Dose-response
- Exposure Assessment
  - Likely intake of pathogen
- Risk Characterization
  - Consequences, given the above

National Research Council (NRC) process for chemical RA

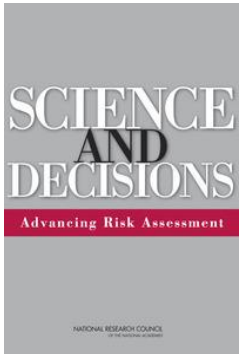
- Hazard Identification
- Dose-Response
- Exposure Assessment
- Risk Characterization



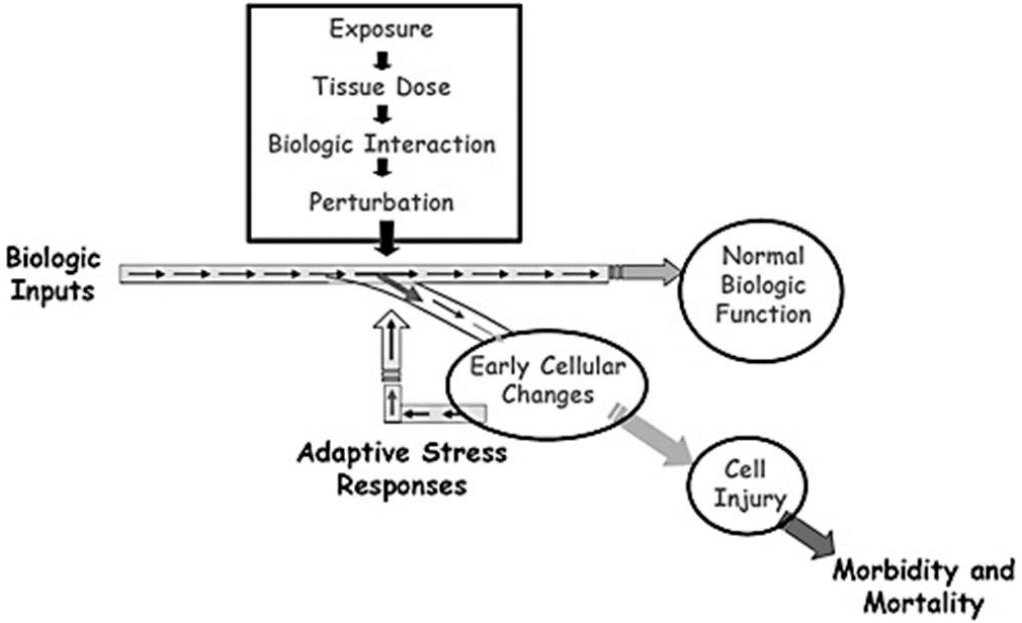
Risk Analysis

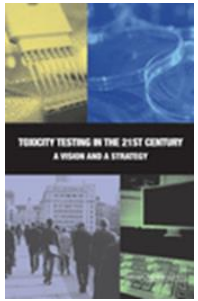


Adapted from NRC, 2009 (National Research Council. 2009. Science and Decisions: Advancing Risk Assessment. National Academies Press, Washington, DC.)



Toxicity Testing in the 21st Century





Toxicity Testing in the 21st Century: A Vision and a Strategy (2007)

FIGURE 2-2 Biologic responses viewed as results of an intersection of exposure and biologic function. The intersection leads to perturbation of biologic pathways. When perturbations are sufficiently large or when the host is unable to adapt because of underlying nutritional, genetic, disease, or life-stage status, biologic function is compromised, and this leads to toxicity and disease.
Adapted from Andersen *et al.* 2005. *Trends in Biotechnology*.

Mode of Action (MOA)

- MOA characterizes a general understanding of how a chemical acts in the body
- MOAs or endpoints caused by a chemical can be evaluated to determine relevance to humans
- MOA can aid in identifying groups in the population or lifestages that may have increased susceptibility
- MOA issues can aid in developing and refining research strategies
- MOA focuses not only on apical effect(s), but also on the sequence of key events towards the endpoints of concern
- Need to identify what tests can be used to inform an MOA evaluation
- Can be used to refine uncertainty factors (UFs) used for determination of health-based guidance values (HBGVs)

Adverse Outcome Pathway (AOP)

- As defined by the OECD, an AOP is "the sequential progression of events from the molecular initiating event (MIE) to the *in vivo* outcome of interest."
- Aim is to integrate knowledge of the interaction of chemicals with biological systems, with particular emphasis on changes causing adverse apical outcomes (for this effort, presume genomic damage).

GTTC Clean Sheet Workgroup

- HESI Genetic Toxicology Technical Committee (GTTC) workgroup:

  "Clean Sheet" Workgroup

- The goal of this workgroup is to develop a genetic toxicology testing strategy from a clean slate, incorporating new science and technology

- Manuscript
  - Next Generation Testing Strategy for Assessment of Genomic Damage: A Conceptual Framework and Considerations
    - Dearfield *et al.*, Environmental and Molecular Mutagenesis 58:264-283 (2017)

"Clean Sheet"

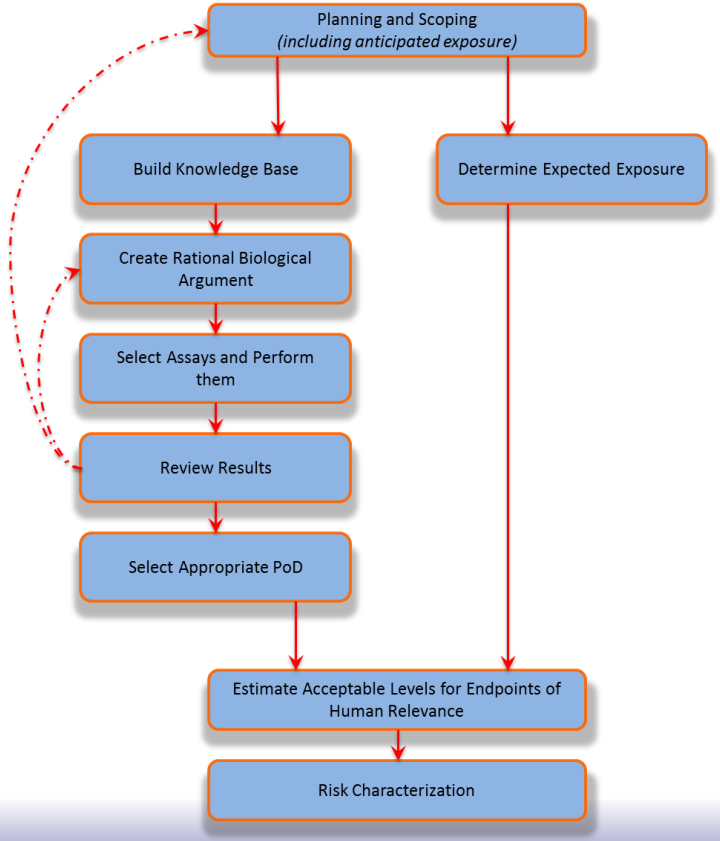
- Current testing strategy no longer the single approach for examining all the aspects of genomic damage and mode of action (MOA)
- Given that the genomic damage can potentially lead to a multitude of apical effects, testing strategies should be integrated and overlapping and take full benefit of the advances in systems biology tools
- Need a testing strategy that is relevant to human risk assessment and provides assurance of safety from chemical exposures in the environment, in the food supply, and in pharmaceutical use

Next Generation Testing Strategy

Conceptual Framework:

1. Planning & Scoping (risk management questions)
2. Build Knowledge Base
3. Create Rational Biological Argument
4. Select Assays and Perform Them
5. Review Results
6. Select Appropriate PODs (dose-response modeling)
7. Determine Expected/Actual Exposures
8. Estimate Candidate Regulatory Levels for Endpoints of Most Concern/Relevance
9. Risk Characterization – address risk management questions

Next Generation Testing Strategy



Planning and Scoping

- Sets up reasons for why you are testing (construct appropriate risk management questions), *e.g.*,
  - Why are you testing?
  - What regulation(s) are you addressing?
  - What exposure(s) do you need to take into account?
    - Environmental, food supply, or pharmaceutical? Other?
    - If no exposure, do you need to test?
  - Who is going to do the testing? What are the logistics? Timeframes?

Exposure-Based Qualitative Categories to Inform Development of a Base Set of Genetic Toxicity Testing Data

Exposure Group	Exposed Population	Exposure-based Category and Expected Actions
Closed system/Isolated intermediate– Industrial use only	Industrial/Production workers only	Minimal/Low exposure; Expect reliable use of recommended Personal Protective Equipment (PPE)
Incorporation into or onto matrix–Industrial use	Industrial workers only	Low exposure; Expect reliable use of recommended PPE
Non-dispersive/Professional use	Professional workers only	Moderate exposure potential; Expect reliable use of recommended PPE; Dose-response data/determination of PoDs provides perspective on potential for risk
Wide dispersive	Environmental/Human populations	Potential for wide exposure in general population; Industrial chemicals, drugs, etc. which are discharged into the environment via waste streams during manufacture or end of life after disposal; No expectations vis-a-vis PPE; Dose-response data/determination of PoDs provides perspective on potential for risk; Will likely conduct risk assessment
Wide dispersive/Consumer use	Consumers	Potential for wide/high exposure in general population; Chemicals such as drugs, devices, and food-based substances for consumer use; No expectations vis-a-vis PPE; Dose-response data/determination of PoDs provides perspective on potential for risk; Will most likely conduct risk assessment

Minimal Information/Data Set

- Regardless of the concern level for any substance, a minimal information/data set is necessary to determine what decisions regarding genomic damage can be made
- This set can provide information as to whether there is the potential for genomic damage that further testing needs to address
- It can also indicate that no further testing is needed such that a risk management decision can be made based on the minimal data set
- Under most RA schemes, “database insufficiency” triggers use of an additional UF. This can be avoided if the minimal set provides “sufficient” information for genotoxicity assessment

Minimal Information/Data Set (cont.)

- For the minimal data set, a starting point would be to use *in silico* methods (*e.g.*, QSAR computational toxicology), short-term, high-throughput assays, and toxicogenomics (maybe even whole genome sequencing) that can provide a broad coverage of potential toxicity pathways
- Or, if for example, the substance of interest is most likely a direct DNA reactive substance based on the knowledge base, then the existing standard regulatory battery may be a reasonable minimal set of tests

Minimal Information/Data Set (cont.)

- The results from the minimal set of information/tests can lead to a presumption of hazard and can provide input to an initial risk management decision:
  - commit to additional testing if results are positive
  - not continue testing to initiate mitigation(s) if positive
  - stop any further work if negative
- This decision also takes into account the initial exposure scenario – if little exposure is expected or seen, then the decision to bring the testing to a close is more likely; whereas, if high and/or widespread exposure is expected, additional testing is likely (unless circumstances dictate immediate action as in an emergency exposure situation)

Build Knowledge Base

- Following along with planning & scoping is the effort to assemble what you know about the compound(s) being considered
  - Intended uses
  - Biological targets (tissues, cell types, intracellular targets)
  - Physico-chemical characteristics
  - (Q)SAR information
  - Analogue information/read across assessment
  - *In silico* assessments
  - ADME information
  - Mode of action (MOA) information
  - Existing test results (any relevant toxicology test)
  - Existing human data

In Silico Approaches

- (Quantitative) Structure–Activity Relationships [(Q)SAR] and analogue information provide predictions of potential chemical toxic activity
- (Q)SAR models can be used for both screening and prioritization
- Provide insights for possible tests to perform
- Analogue searching (read-across) is a method that identifies similar chemicals to the one of interest, not only in terms of chemical structure, but specifically also with a similar functionality which is related to the toxicological effect of interest

In Silico Approaches

Examples of Existing (Q)SAR Software Systems

- CASE Ultra
- Derek Nexus and Sarah Nexus
- Model Applier
- Symmetry
- OECD Toolbox (freely available)

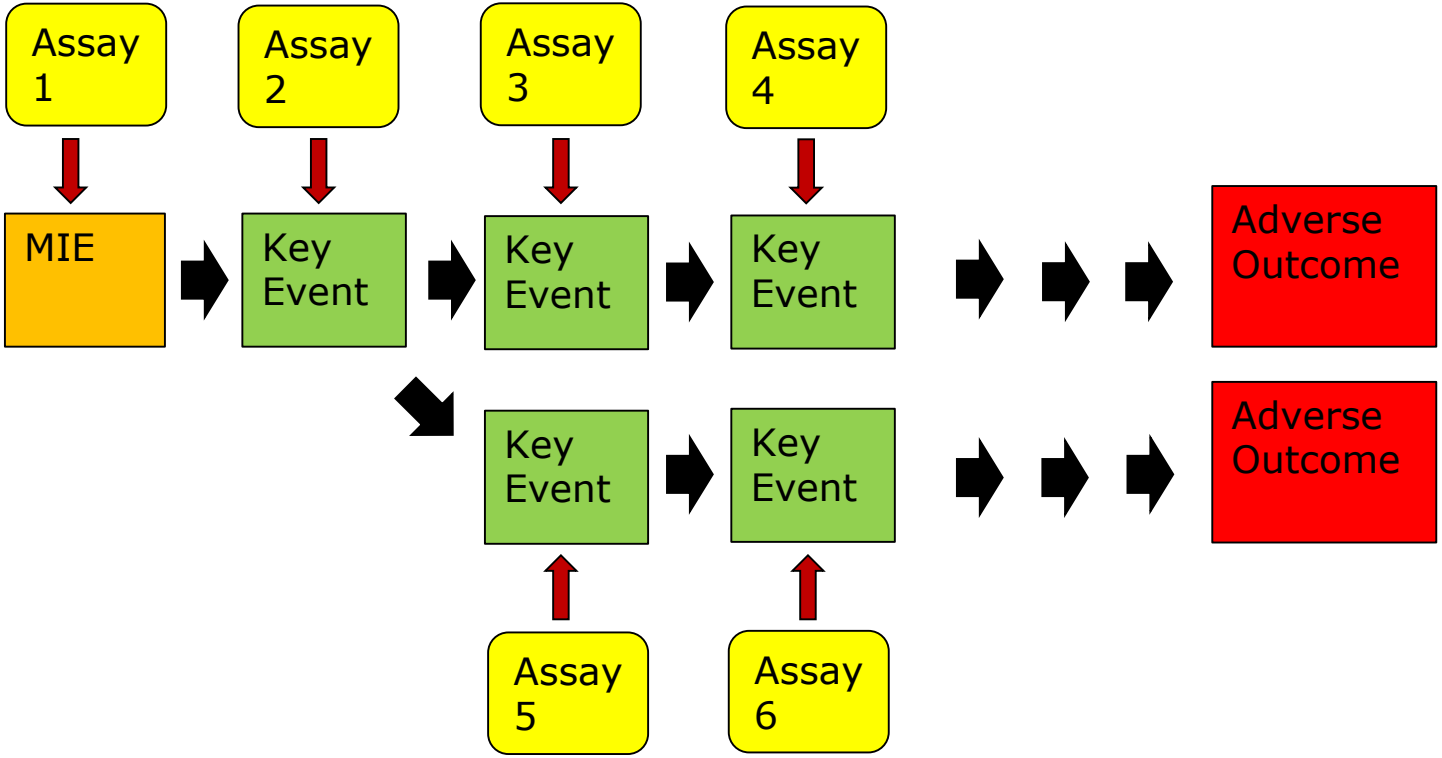
(Q)SAR prediction is accepted in the REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) regulatory framework as an input into a Weight of Evidence, where model prediction, for example together with *in vitro* information, can be sufficiently convincing to replace (waive) *in vivo* testing.

ICH M7 on impurities: use of 2 *in silico* systems (if exposure $\leq$ 1 mg/kg)

Create Rational Biological Argument

- Build your argument for what specific testing strategy you propose to use for your specific compound based on your knowledge base
- Can look to mode of action for possible toxicity pathways to test against
- Can look to AOPs for guidance on where to test

Adverse Outcome Pathway (AOP)



Select Assays and Perform Them

- Assays will be pertinent to your proposed mode of action
- Can be a few tests, or several tests
- May include non-genotoxicity tests
- Likely be *in vitro* at first, but can include *in vivo* as need arises
- See about high throughput type tests
  - Set of “sentinel” high through-put assays that provide “markers” or “flags” for specific toxicity pathways or AOPs

Example Technologies to Consider

- Single cell gel electrophoresis (SCGE) or comet assay (directly measures DNA damage in individual cells)
- Pig-a assay (measures the frequency of cells without specific surface markers due to mutations at the *Pig-a* gene)
- Transgenics (use of a transgenic animal model with marker, *e.g.*, lac z, lac I, cII)
- Immunofluorescent antikinetochore (CREST) & fluorescence *in situ* hybridization (FISH) assays (*e.g.*, help distinguish micronuclei formed from chromosome loss from those originating from chromosome breakage)
- Toxicology Testing in the 21st Century (Tox21) (developed and validated *in vitro* cell-based assays (tests) using quantitative high-throughput screening to test whether certain chemical compounds have the potential to disrupt processes in the human body that may lead to negative health effects; use of computational research with assay results (*e.g.*, ToxCast™); Collaboration among EPA, NIH's NCATS and NTP, and FDA)

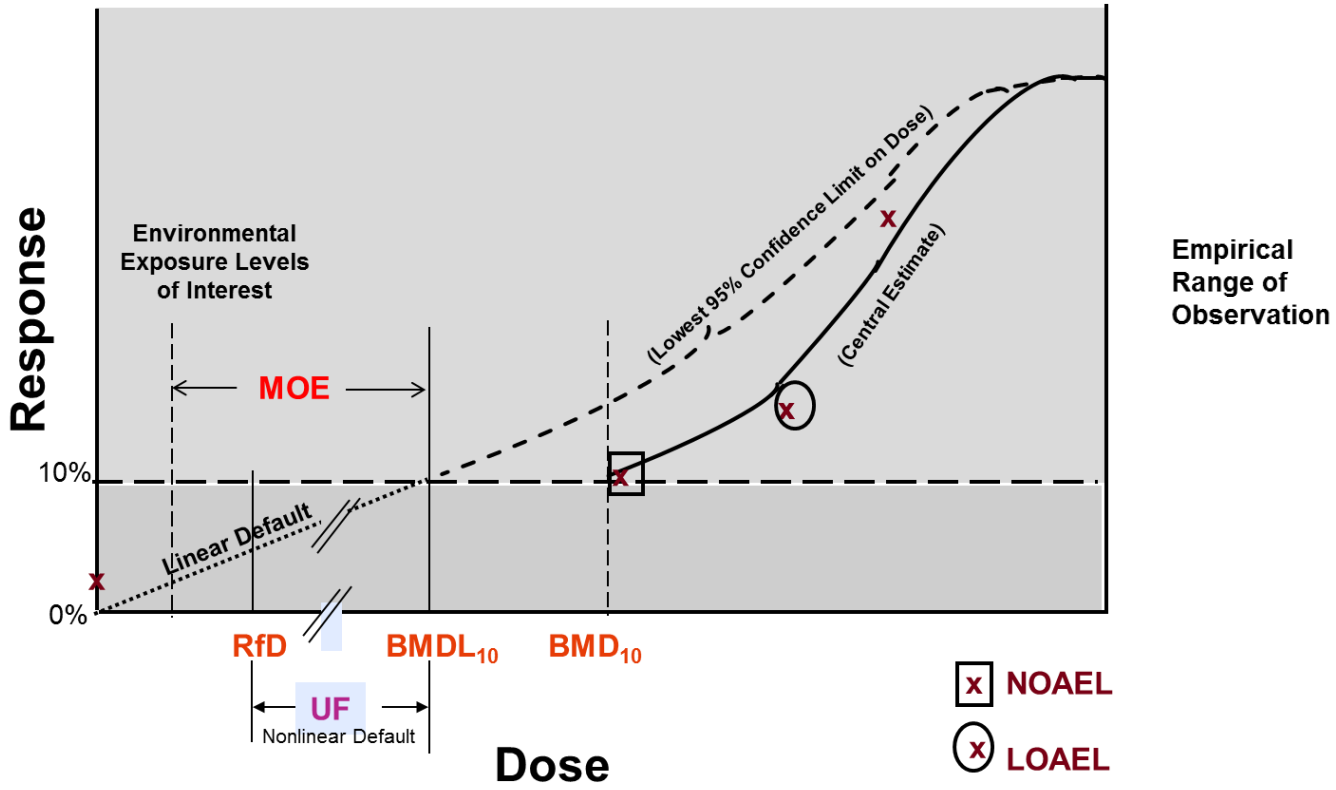
Example Technologies to Consider

- Flow Cytometry methods (*e.g.*, study micronuclei induction; study multiple endpoints associated with DNA damage response pathways (MultiFlow™))
- Toxicogenomics (using omics-based biomarkers, *e.g.*, transcriptomic biomarker TGx-28.65; look at gene expression profiles of *in vitro* exposed cell systems; can integrate into almost any assay)
- Next Generation Sequencing (NGS)
- ToxTracker® (an *in vitro* mammalian stem cell-based reporter assay that detects activation of specific cellular signaling pathways upon exposure to unknown compounds; detect by flow cytometry fluorescent reporters for points in signaling pathways)
- 3D culture products (to emulate cellular behaviors and morphologies similar to those seen *in vivo*; organ or tissue models; combination assay model (skin comet and MN assays); multiorgan chip (circulatory system + urine flow); 3D spheroids (HepaRG cell spheroids))

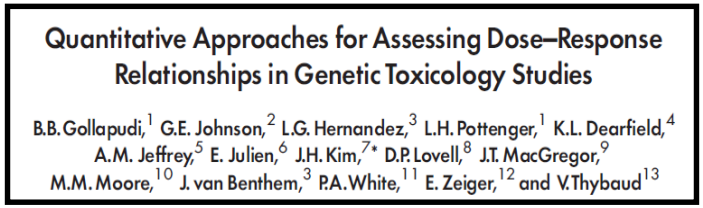
After Testing

- Review results
- Determine which test results to use for determining a point of departure (POD) for risk management purposes
- Use quantitative approaches for dose-response modeling
- Bring in expected/actual human exposure(s)

Low Dose Extrapolation

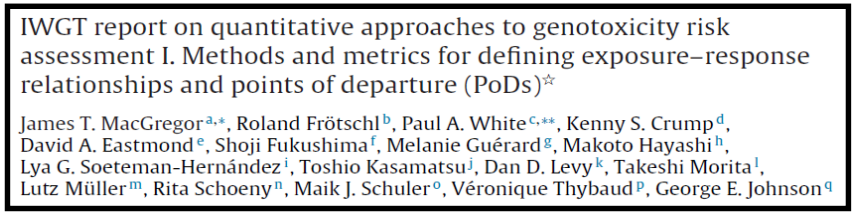


Quantitative Analysis


Quantitative Approaches for Assessing Dose–Response Relationships in Genetic Toxicology Studies

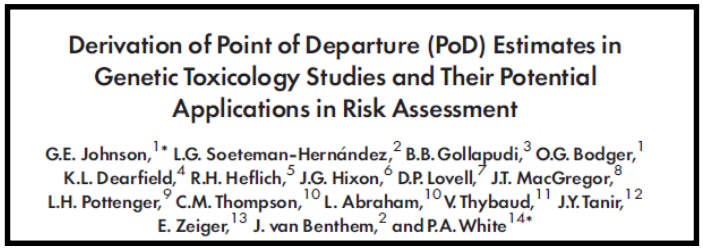
B.B. Gollapudi,[1] G.E. Johnson,[2] L.G. Hernandez,[3] L.H. Pottenger,[1] K.L. Dearfield,[4] A.M. Jeffrey,[5] E. Julien,[6] J.H. Kim,[7]* D.P. Lovell,[8] J.T. MacGregor,[9] M.M. Moore,[10] J. van Benthem,[3] P.A. White,[11] E. Zeiger,[12] and V. Thybaud[13]

Gollapudi *et al.*, 2013. *Environmental and Molecular Mutagenesis* 54:8-18.


IWGT report on quantitative approaches to genotoxicity risk assessment I. Methods and metrics for defining exposure–response relationships and points of departure (PoDs)☆

James T. MacGregor[a,*], Roland Frötschl[b], Paul A. White[c,**], Kenny S. Crump[d], David A. Eastmond[e], Shoji Fukushima[f], Melanie Guérard[g], Makoto Hayashi[h], Lya G. Soeteman-Hernández[i], Toshio Kasamatsu[j], Dan D. Levy[k], Takeshi Morita[l], Lutz Müller[m], Rita Schoeny[n], Maik J. Schuler[o], Véronique Thybaud[p], George E. Johnson[q]

MacGregor *et al.*, 2015. *Mutation Research*, 783:66-78.


Derivation of Point of Departure (PoD) Estimates in Genetic Toxicology Studies and Their Potential Applications in Risk Assessment

G.E. Johnson,[1]* L.G. Soeteman-Hernández,[2] B.B. Gollapudi,[3] O.G. Bodger,[1] K.L. Dearfield,[4] R.H. Heflich,[5] J.G. Hixon,[6] D.P. Lovell,[7] J.T. MacGregor,[8] L.H. Pottenger,[9] C.M. Thompson,[10] L. Abraham,[10] V. Thybaud,[11] J.Y. Tanir,[12] E. Zeiger,[13] J. van Benthem,[2] and P.A. White[14]*

Johnson *et al.*, 2014. *Environmental and Molecular Mutagenesis* 55:609-623.


IWGT report on quantitative approaches to genotoxicity risk assessment II. Use of point-of-departure (PoD) metrics in defining acceptable exposure limits and assessing human risk☆

James T. MacGregor[a,*], Roland Frötschl[b], Paul A. White[c], Kenny S. Crump[d], David A. Eastmond[e], Shoji Fukushima[f], Melanie Guérard[g], Makoto Hayashi[h], Lya G. Soeteman-Hernández[i], George E. Johnson[j], Toshio Kasamatsu[k], Dan D. Levy[l], Takeshi Morita[m], Lutz Müller[g], Rita Schoeny[n], Maik J. Schuler[o], Véronique Thybaud[p]

MacGregor *et al.*, 2015. *Mutation Research*, 783:55-65.

Detailed scrutiny of data analysis options highlighted the BMD approach as the most robust and pragmatic

Risk Management Options

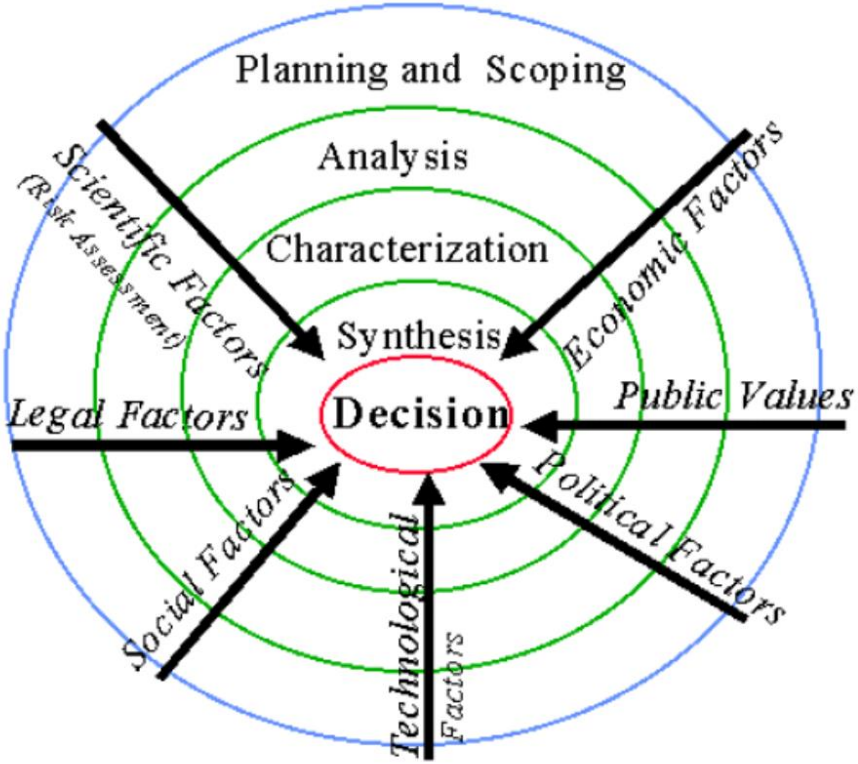
- Then use POD to help extrapolate to lower doses; can use a reference dose (RfD) approach or a margin of exposure (MOE) approach

Assay Result	POD	Exposure	MOE
a	x	Ex1	100,000
b	y	Ex1	1000
c	z	Ex1	10
Etc.	…	…	…

Risk Characterization

- Provide risk managers with estimates of genetic risk, *i.e.*, provides assessment for genomic damage
- In the context of genomic damage, helps address risk management questions posed during planning & scoping, *e.g.*:
  - Can the chemical be used safely if being registered?
  - Does the presence and/or amount of chemical make a food adulterated?
  - Will there be side effects from intended use?

Decision-Making Framework



From:
EPA Risk Characterization Handbook (2000)

To Sum Up

- Toxicology is using a risk assessment approach to determine potential risk to exposed humans (beyond the hazard id approach the genetox community mostly uses now)
- Regulatory agencies now can embrace a more flexible, broader approach for assessing genomic damage and its implication(s) for adverse health outcomes
- This approach applies to any chemical to which humans are exposed, including pharmaceuticals, agricultural products, food additives, and other chemicals

The End

Thank you very much

Any questions?

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The mention of commercial products, their sources, or their use in connection with material presented herein is not to be construed as either an actual or implied endorsement of such products.

Abstract Summary

For decades, testing for genetic toxicity or genomic damage followed a standard set of tests for regulatory purposes. This standard set usually examined mutations and structural and numerical chromosome damage performed with validated, well recognized assays (e.g., Ames test, micronucleus test). For risk assessment purposes, the results from such testing focused mostly on hazard identification and whether the substance being tested should be a candidate for carcinogenicity testing. Genetic toxicology testing is currently shifting from this yes/no approach for genomic damage potential to encompass the full risk assessment approach (including dose-response analysis, exposure, and characterization of the risk) to more fully determine human genetic toxicity risk from a potential exposure to a substance. This shift is occurring as the knowledge about how genomic damage is induced has greatly expanded from the electrophilic mechanisms of early tested well known mutagens, to a whole array of modes of action for genomic damage. Genetic toxicology assessment needs to grow with this expanding knowledge and allow for a more flexible approach to testing for genomic damage. Newer tests for genomic damage are focusing more on toxicity pathways, suspected modes of action, and higher throughput. These approaches combined with a better assessment of potential exposures make for a more relevant assessment of human genetic toxicity risk.