NexGen:
The Next Generation of Risk Science

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Next generation risk assessment

Three Cornerstones

- New paradigm for toxicity testing (TT21C), based on perturbation of toxicity pathways

- Advanced risk assessment methodologies, including those addressed in *Science and Decisions*

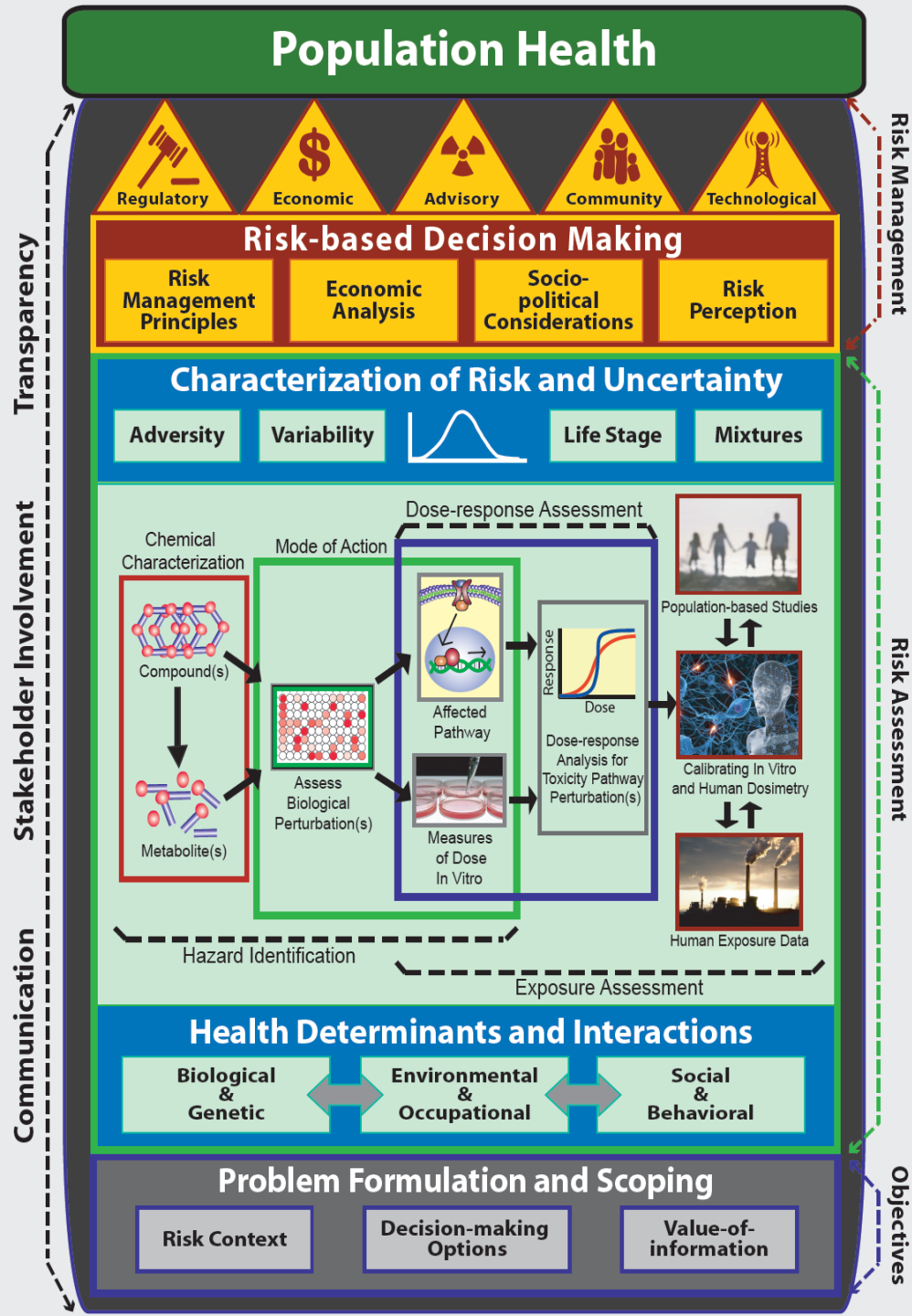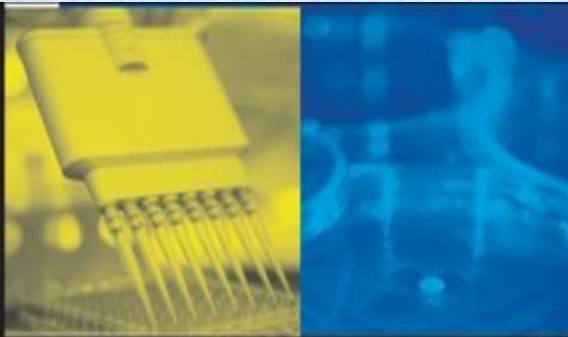- Population health approach: multiple health determinants and multiple interventions

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TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND STRATEGY

Chemical Characterization

Risk Contexts

Toxicity Testing

Toxicity Pathways   Targeted Testing

Population and Exposure Data

Dose-Response and Extrapolation Modeling
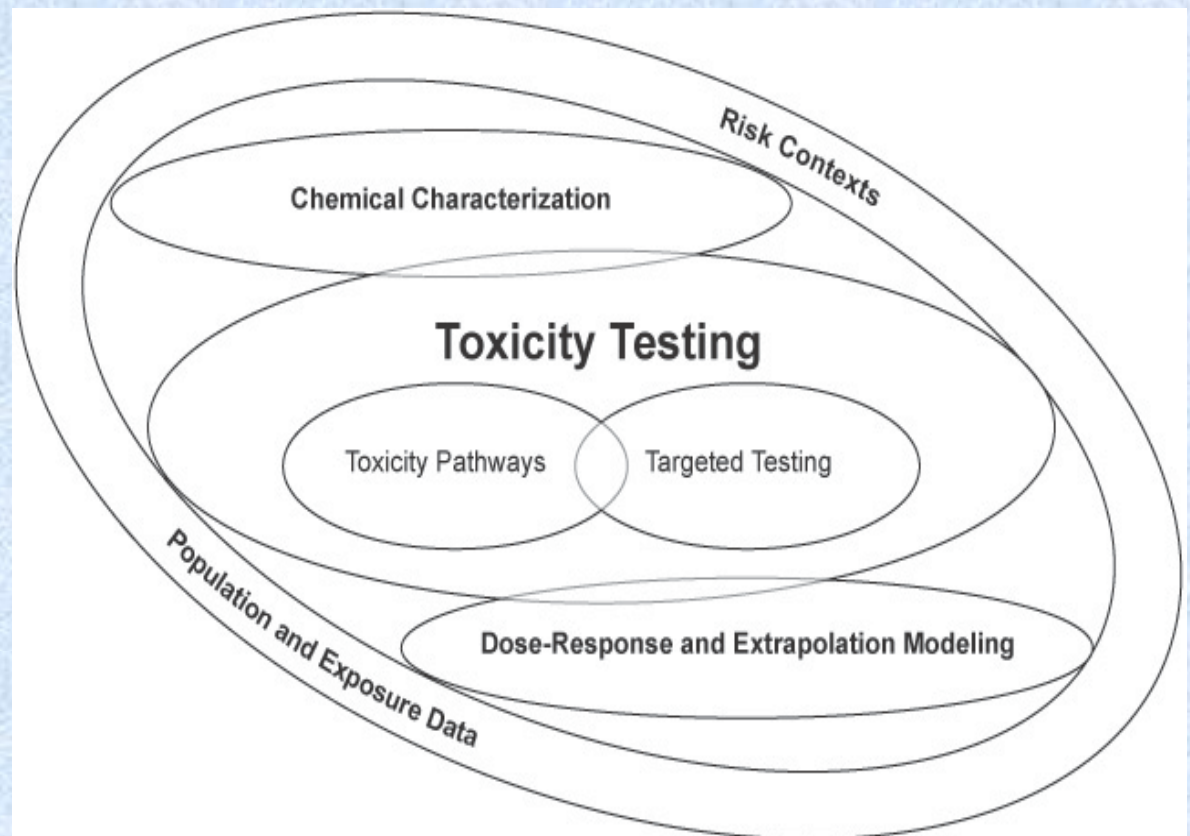
www.nas.edu

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Building the Scientific Toolbox
(Andersen & Krewski, 2009, Tox. Sci)

Tool	Application
High throughput screens	Efficiently identify critical toxicity pathway perturbations across a range of doses and molecular and cellular targets
Stem cell biology	Develop in vitro toxicity pathway assays using human cells produced from directed stem cell differentiation
Functional genomics	Identify the structure of cellular circuits involved in toxicity pathway responses to assist computational dose response modeling
Bioinformatics	Interpret complex multivariable data from HTS and genomic assays in relation to target identification and effects of sustained perturbations on organs and tissues
Systems biology	Organize information from multiple cellular response pathways to understand integrated cellular and tissue responses
Computational systems biology	Describe dose-response relationships based on perturbations of cell circuitry underlying toxicity pathway responses giving rise to thresholds, dose-dependent transitions, and other dose-related biological behaviors
Physiologically-based pharmacokinetic models	Identify human exposure situations likely to provide tissue concentrations equivalent to in vitro activation of toxicity pathways
Structure-activity relationships	Predict toxicological responses and metabolic pathways based on the chemical properties of environmental agents and comparison to other active structures
Biomarkers	Establish biomarkers of biological change representing critical toxicity pathway perturbations

Human Toxicology Project Consortium

Accelerating Implementation of the NRC Vision for Toxicity Testing in the 21st Century

A Human Toxicology Project Consortium Symposium

November 9 - 10, 2010

Kellogg Conference Center, Gallaudet University
Washington, DC

EPA/100/K-09/001 | March 2009
www.epa.gov/osa

The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals

Office of the Science Advisor
Science Policy Council

Hamburg, M. (2011), Science (Editorial). Vol. 335. p. 987
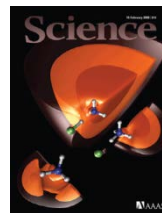
Collins, F.S., Gray, G.M. & Bucher, J.R. (2008), Science (Policy Forum). Vol. 319. pp. 906 - 907
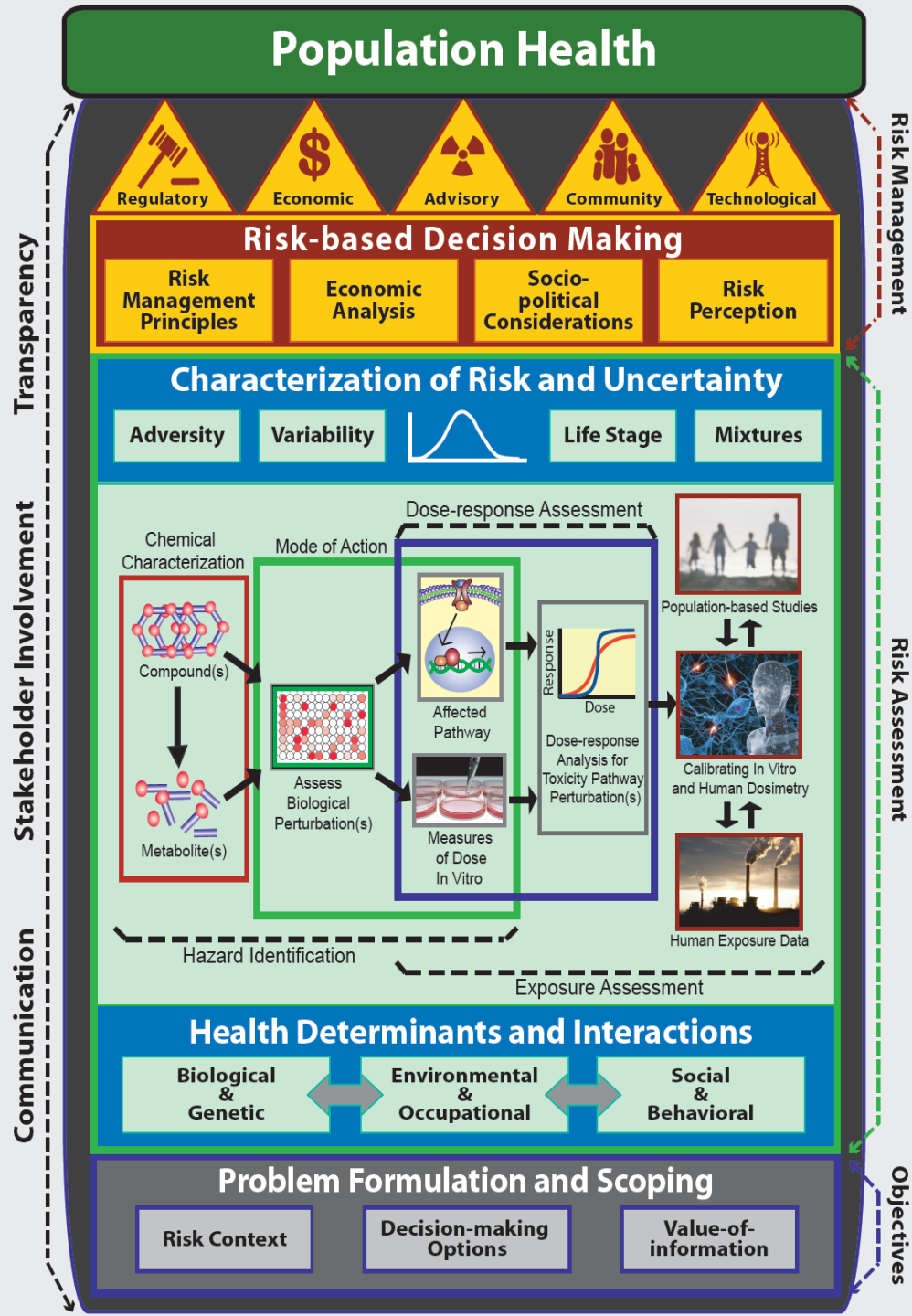
Chemical Characterization
- Compound(s)
- Metabolite(s)

Mode of Action

Dose-response Assessment

Assess Biological Perturbation(s)

Affected Pathway

Measures of Dose In Vitro

Dose-response Analysis for Toxicity Pathway Perturbation(s)

Response / Dose

Population-based Studies

Calibrating In Vitro and Human Dosimetry

Human Exposure Data

Human Exposure Guidelines

Hazard Identification

Exposure Assessment

Risk Characterization

Krewski et al. (2011), ARPH

Three Cornerstones

- New paradigm for toxicity testing (TT21C), based on perturbation of toxicity pathways

- Advanced risk assessment methodologies, including those addressed in *Science and Decisions*

- Population health approach: multiple health determinants and multiple interventions

- Enhanced framework
- Formative focus
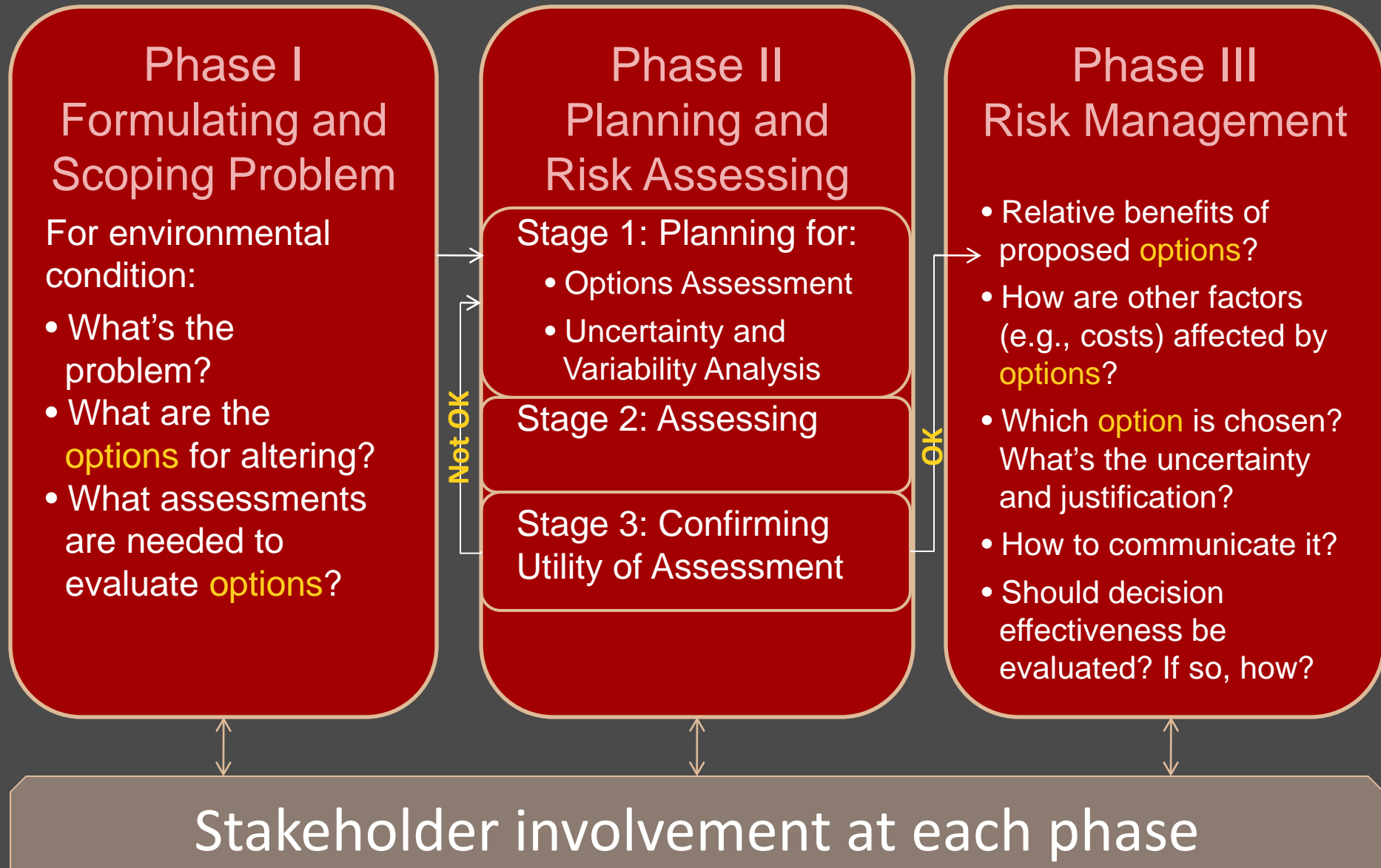- Four steps still core
- Matching analysis to decisions
- Clearer estimates of population risk
- Advancing cumulative assessments
- People and capacity building

"Risk-Based Decision-Making" Framework

Phase I
Formulating and Scoping Problem

For environmental condition:

- What's the problem?
- What are the options for altering?
- What assessments are needed to evaluate options?

Phase II
Planning and Risk Assessing

Stage 1: Planning for:
- Options Assessment
- Uncertainty and Variability Analysis

Stage 2: Assessing

Stage 3: Confirming Utility of Assessment

Not OK

OK

Phase III
Risk Management

- Relative benefits of proposed options?
- How are other factors (e.g., costs) affected by options?
- Which option is chosen? What's the uncertainty and justification?
- How to communicate it?
- Should decision effectiveness be evaluated? If so, how?

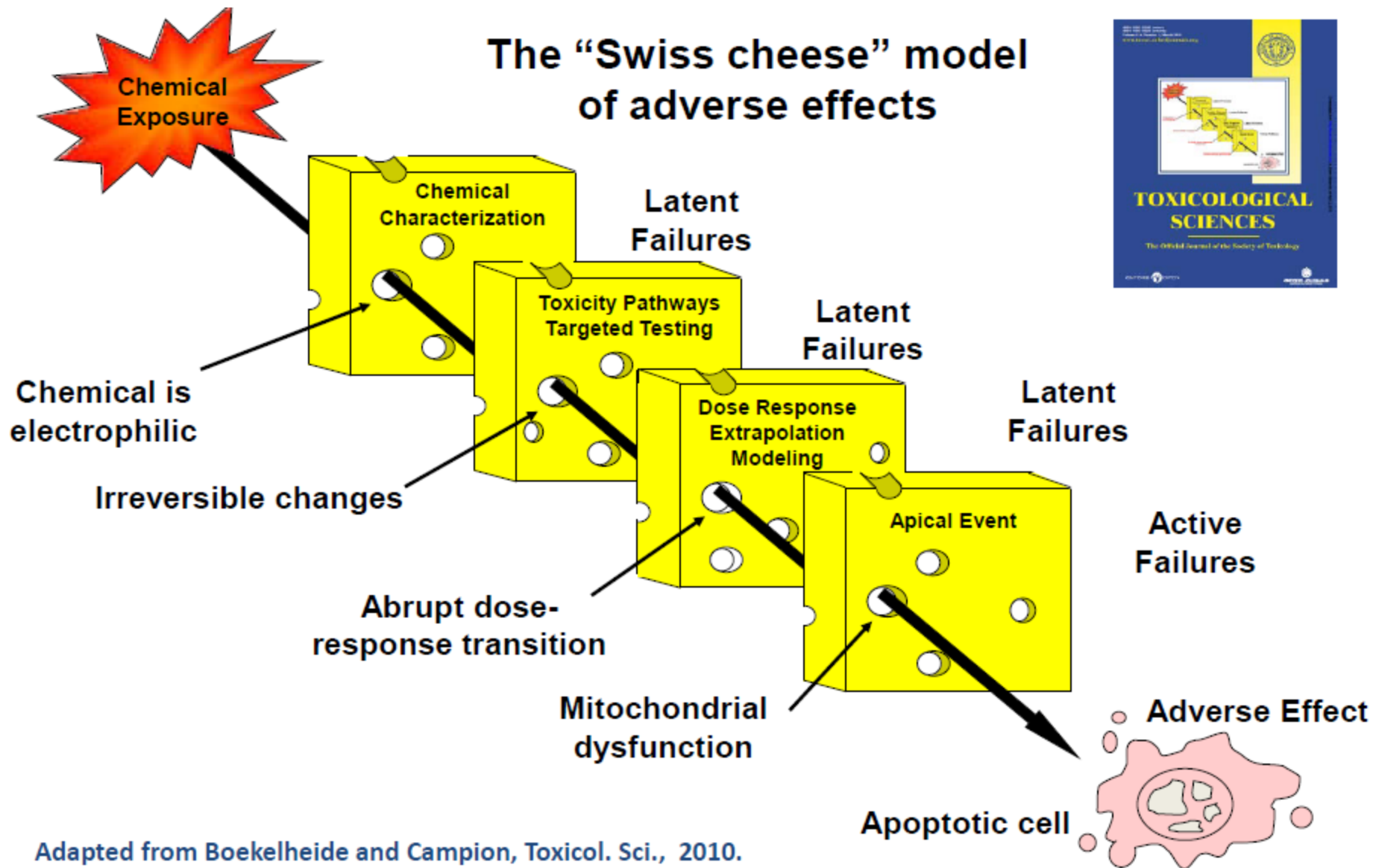## Stakeholder involvement at each phase

Methodological Issues

- Adversity
- Variability
- Susceptible populations
- Dose and species extrapolation
- Mixtures and multiple stressors
- Uncertainty analysis

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Methodological Issues: (1) Adversity

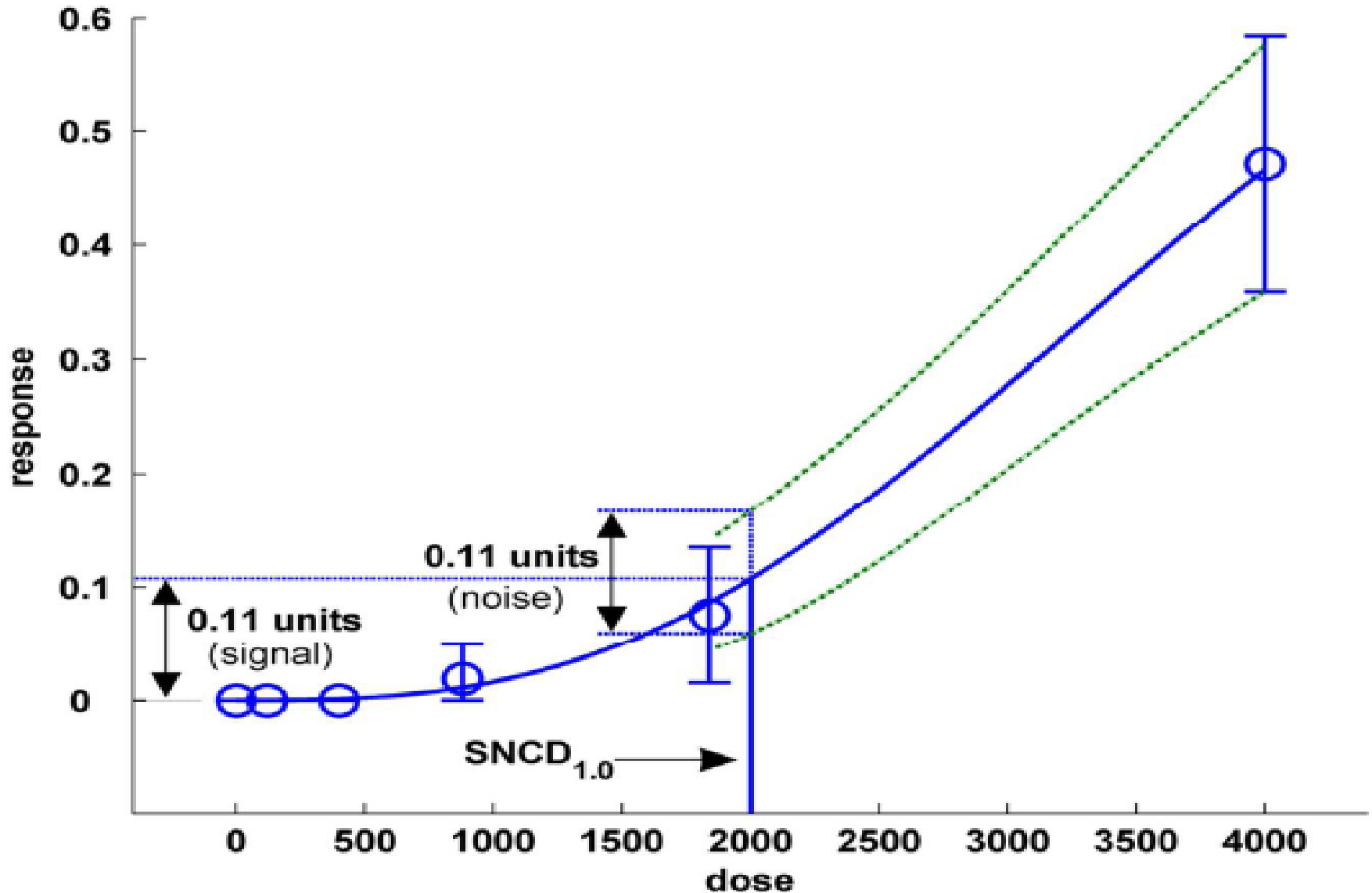| Issue	Current Approach	NexGen Approach
Adverse outcomes	Apical outcomes in mammalian systems, or precursors to these outcomes, serve as the basis for risk assessment.	In vitro assays identify critical toxicity pathway perturbations, which serve as the basis for risk assessment, even in the absence of a direct link with an apical outcome.

The "Swiss cheese" model of adverse effects

Adapted from Boekelheide and Campion, Toxicol. Sci., 2010.

Methodological Issues: (2) Dose-response

Issue	Current Approach	NexGen Approach
Dose-response assessment	Empirical or biologically-based models describe apical endpoints, and determine an appropriate point of departure (such as the benchmark dose) for establishing a reference dose.	Computational systems biology pathway models describe dose-response relationships for pathway perturbations, reflecting dose-dependent transitions throughout the dose range of interest.

Signal-to-Noise Crossover Dose (SNCD)

Sand, Portier & Krewski (2011), EHP

Methodological Issues: (3) Variability

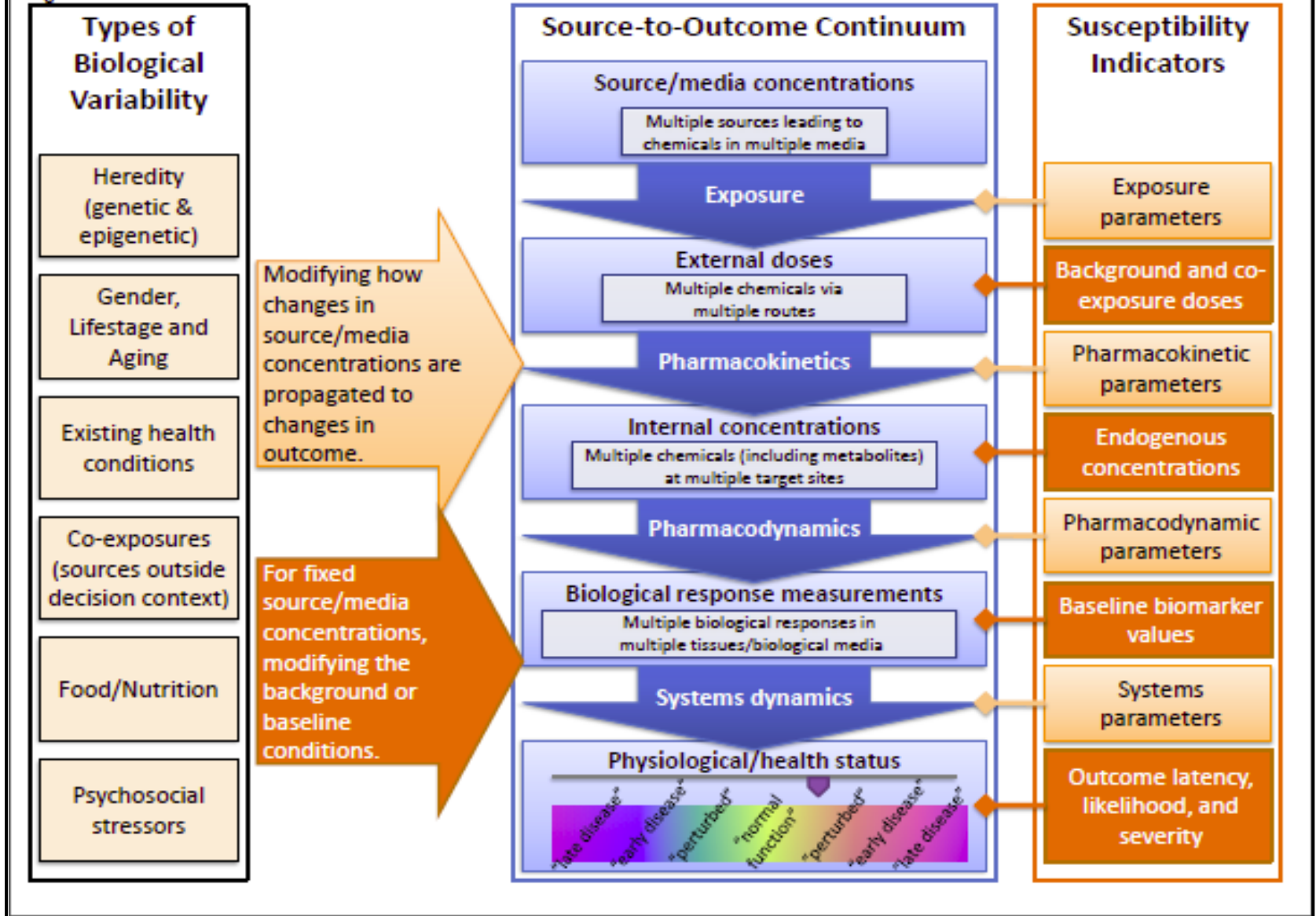| Issue	Current Approach	NexGen Approach
Inter-individual variability	Adjustment factors used in establishing reference doses account for inter-individual variability in PK and PD. Variability in exposure is also taken into account.	Variability in biological response is characterized through the use of a diverse range of human cell lines. Dosimetry models link variation in human exposure with corresponding in vitro doses.

Table 1. Examples of data sources for modeling PK and PD variability

Variability in human phase I and phase II metabolism and renal excretion, including in different age groups - neonates, children and the elderly	(Dorne 2010; Ginsberg et al. 2004; Ginsberg et al. 2002; Hattis et al. 2003)
Compilations of genetic polymorphisms of specific metabolic enzyme activities:	
• paraoxonase	(Ginsberg et al. 2009a)
• N-Acetyltransferase 1 and 2	(Bois et al. 1995; Walker et al. 2009)
• glutathione transferases	(Ginsberg et al. 2009b)
• CYP2D6	(Neafsey et al. 2009b)
• CYP2E1	(Neafsey et al. 2009a)
• ALDH2	(Ginsberg et al. 2009c)
Human biomonitoring observations of inter-individual differences in biomarkers of exposure (e.g., chemical-protein adducts) or in levels of parent/metabolite	(Bois et al. 1996)
Variability in physiological parameters for older adults: bodymass, surface area, body mass index, health status	(Thompson et al. 2009)
Indicators of PD variability such as in:	
• human DNA repair enzyme XRCC1	(Ginsberg et al. 2011)
• human host defense enzymes	(Ginsberg et al. 2010)
• lung function response to particulate matter	(Hattis et al. 2001)
• susceptibility to infectious organisms	(Hattis 1997)

From Zeise et al. (2012): "Assessing Human Variability in the Next Generation Health Assessments of Environmental Chemicals"

Methodological Issues: (4) Susceptibility

Issue	Current Approach	NexGen Approach
Susceptible populations	Life-stage, genetics, and socioeconomic and lifestyle factors determine susceptible population groups.	Molecular and genetic epidemiology defines susceptible populations in terms of critical pathway perturbations.

How susceptibility arises from variability
(from Zeise et al., 2012)

Methodological Issues: (5) Extrapolation

Issue	Current Approach	NexGen Approach
Dose and species extrapolation	Dose and species extrapolation translate animal test results to humans.	Cellular assays provide direct measures of toxicity pathway perturbations in humans. IVIVE techniques and pathway modeling calibrate in vitro and in vivo exposures. Sensitive in vitro tests are used to evaluate risk directly at environmental exposure levels.
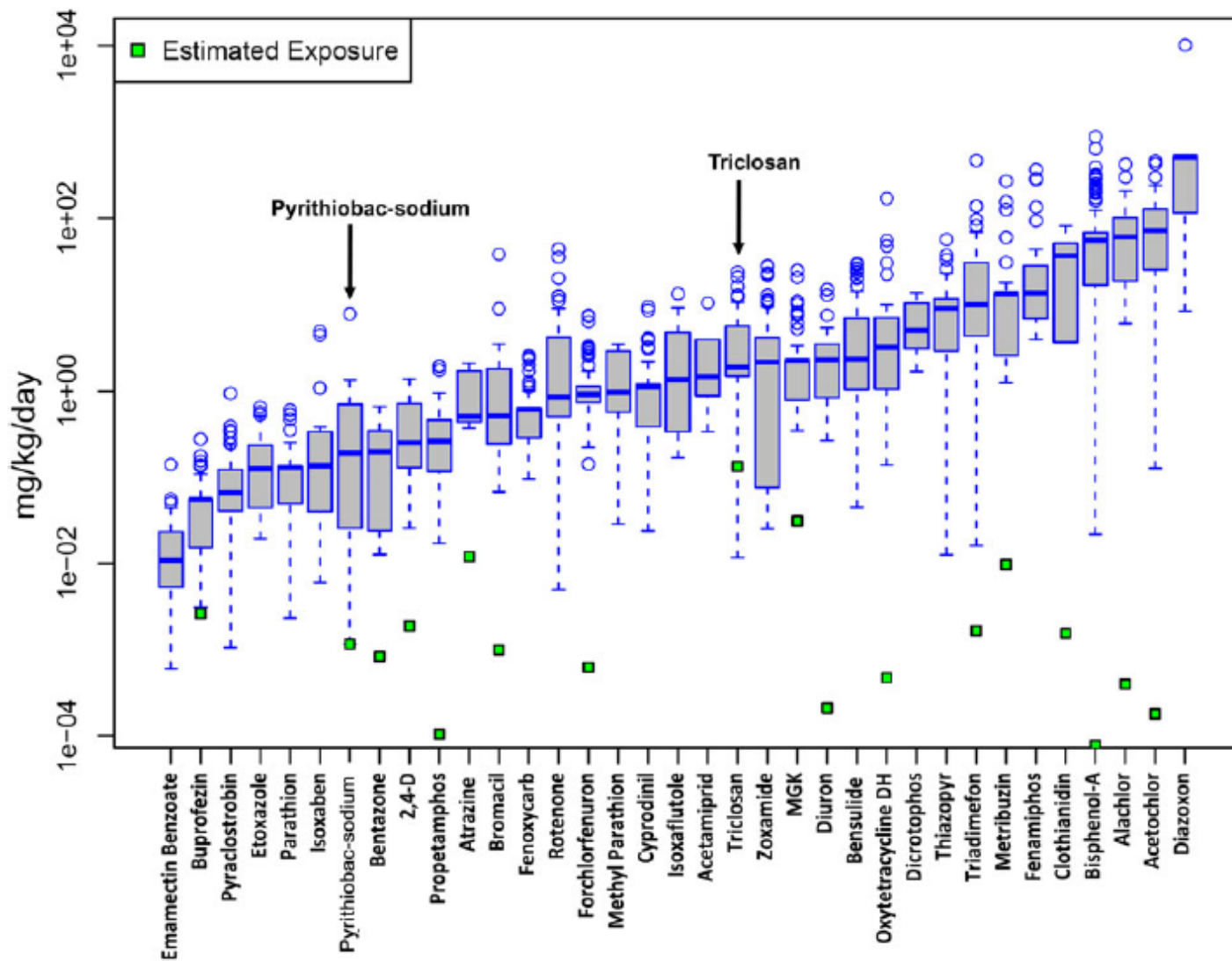
Rotroff DM, Wetmore BA, Dix DJ, Ferguson SS, Clewell HJ, Houck KA, Lecluyse EL, Andersen ME, Judson RS, Smith CM, Sochaski MA, Kavlock RJ, Boellmann F, Martin MT, Reif DM, Wambaugh JF, Thomas RS (2010) Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening. Toxicol Sci 117: 348-358

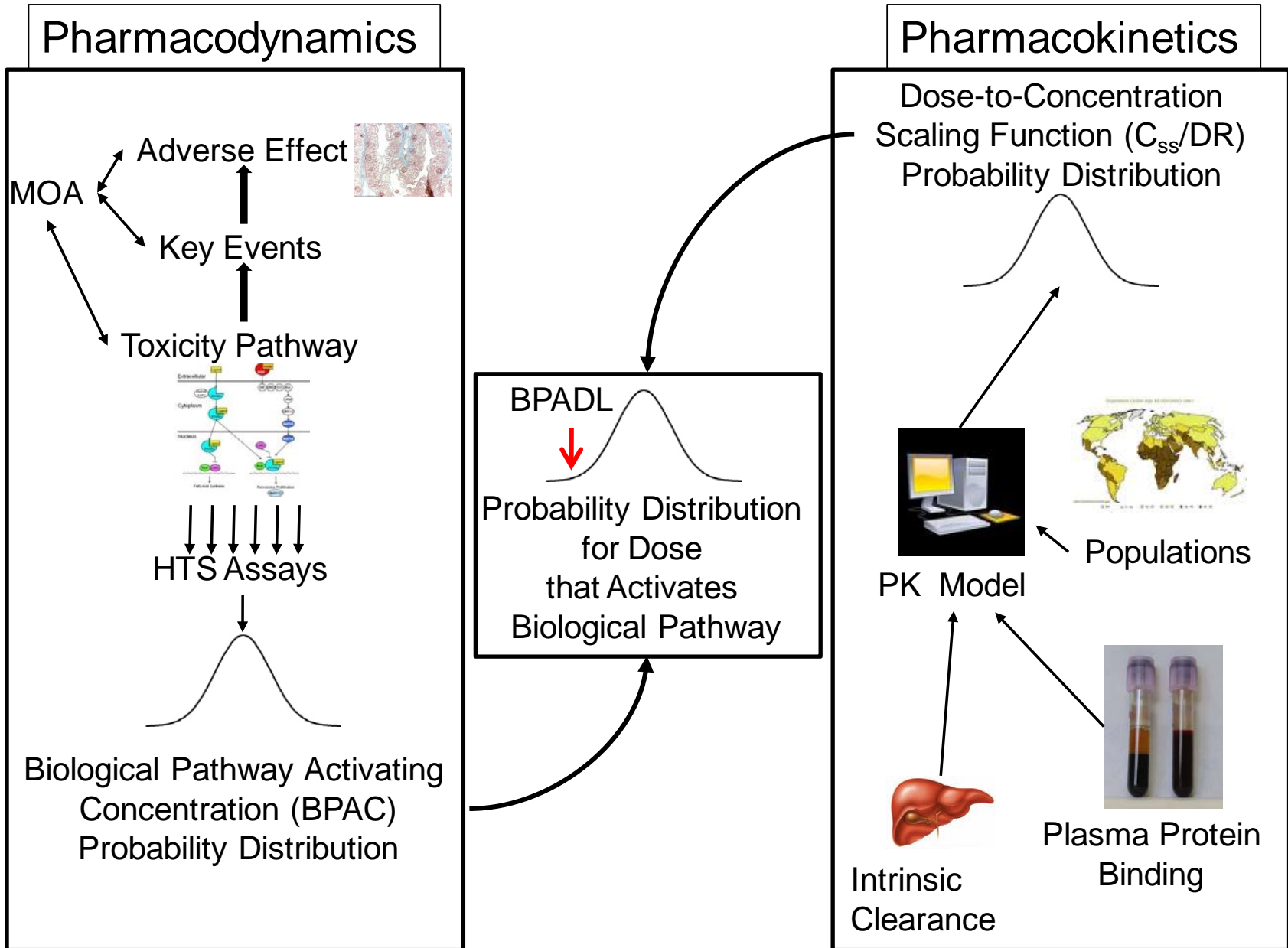# Methodological Issues: (6) Mixtures

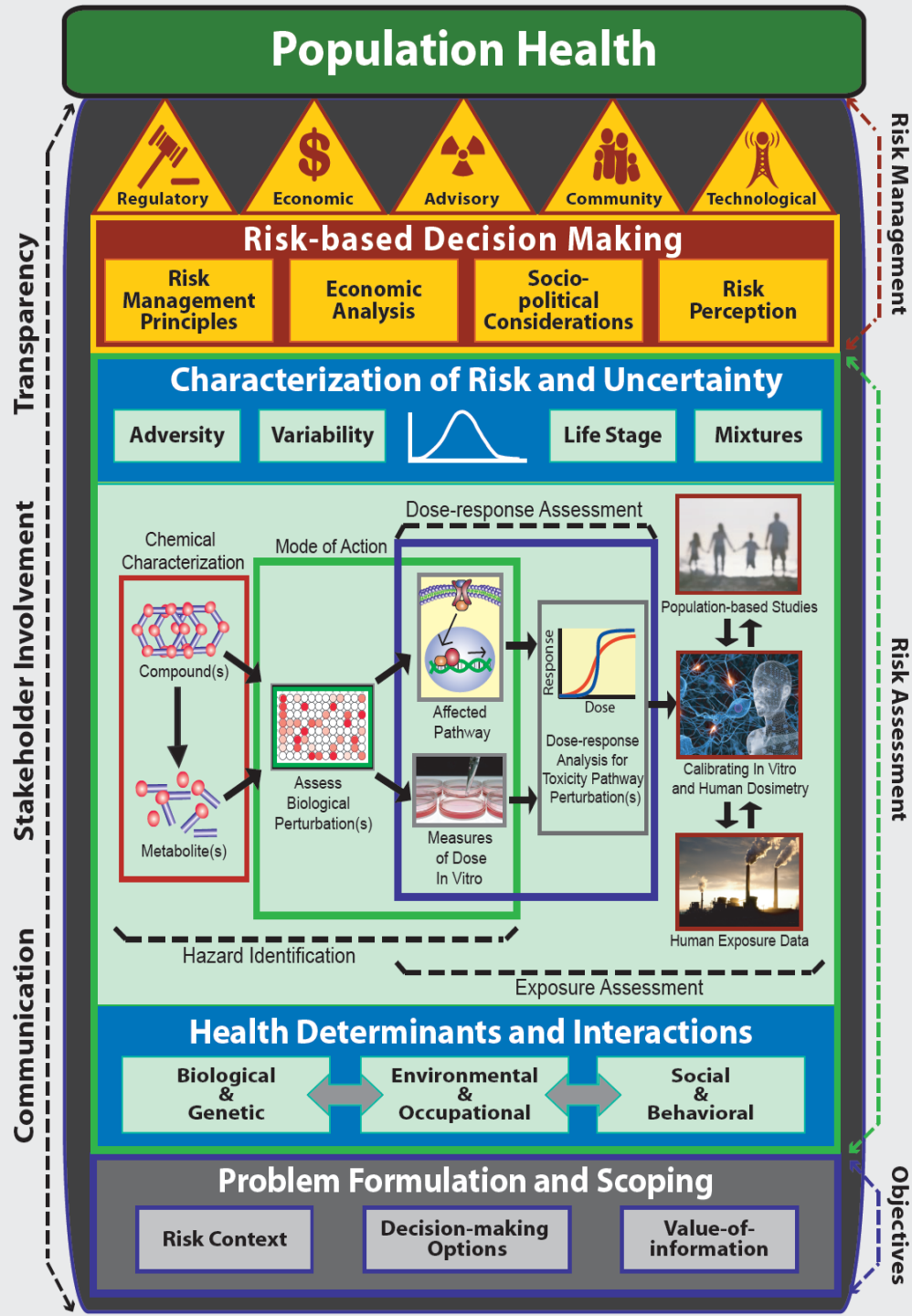| Issue	Current Approach	NexGen Approach
Mixtures and multiple stressors	Common experimental protocols include testing of mixtures and factorial experiments with joint exposures. However, there are only a limited number of such studies because of cost and complexity of experimental design.	Cost-effective high throughput technologies permit expanded testing of mixtures and multiple stressors.

Methodological Issues: (7) Uncertainty

Issue	Current Approach	NexGen Approach
Uncertainty analysis	Uncertainty considerations include species differences in susceptibility, low-dose and route-to-route extrapolation, and exposure ascertainment.	Probabilistic risk assessments characterize overall uncertainty, and identify the most important sources of uncertainty that guide value-of-information decisions.

Reverse Toxicokinetics (rTK):
in vitro concentration to in vivo dose

Pharmacodynamics

MOA

Adverse Effect

Key Events

Toxicity Pathway

HTS Assays

Biological Pathway Activating
Concentration (BPAC)
Probability Distribution

BPADL

Probability Distribution
for Dose
that Activates
Biological Pathway

Pharmacokinetics

Dose-to-Concentration
Scaling Function (C$_{ss}$/DR)
Probability Distribution

PK Model

Populations

Intrinsic
Clearance

Plasma Protein
Binding

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Population Health

| Regulatory | Economic | Advisory | Community | Technological |

← Multiple Interventions →

Health Risk Policy Analysis
Evidence Based Policy

Health Risk Science
Determinants and Interactions

Biology-environment interactions

Environment-social interactions

| Biology and Genetics | Environment and Occupation | Social and Behavioural |

Biology-social interactions
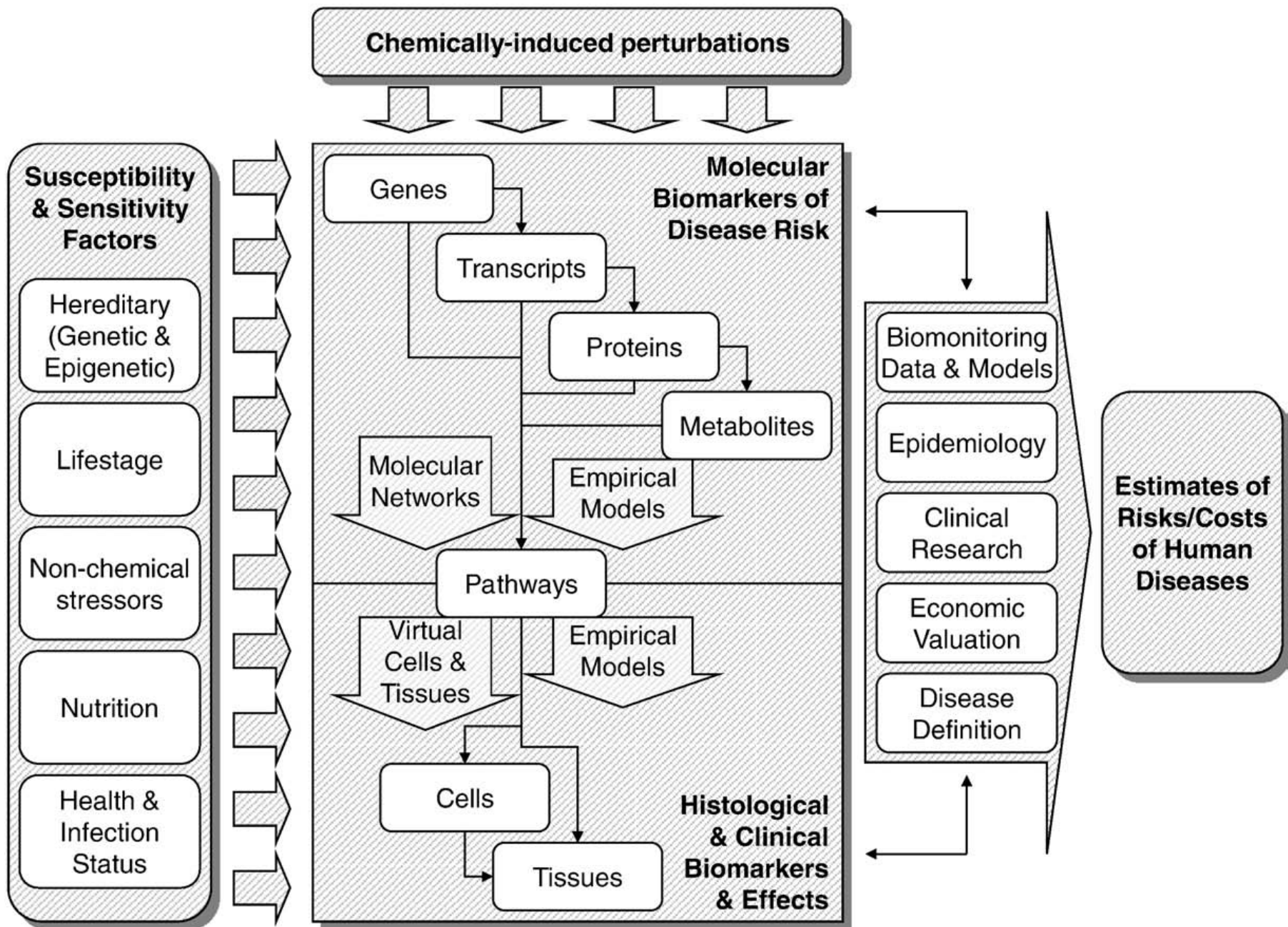
Chiu, W.A., et al., Approaches to advancing quantitative human health risk assessment of environmental chemicals in the post-genomic era, Toxicol. Appl. Pharmacol. (2010), doi:10.1016/j.taap.2010.03.019

Social-Environment Interaction
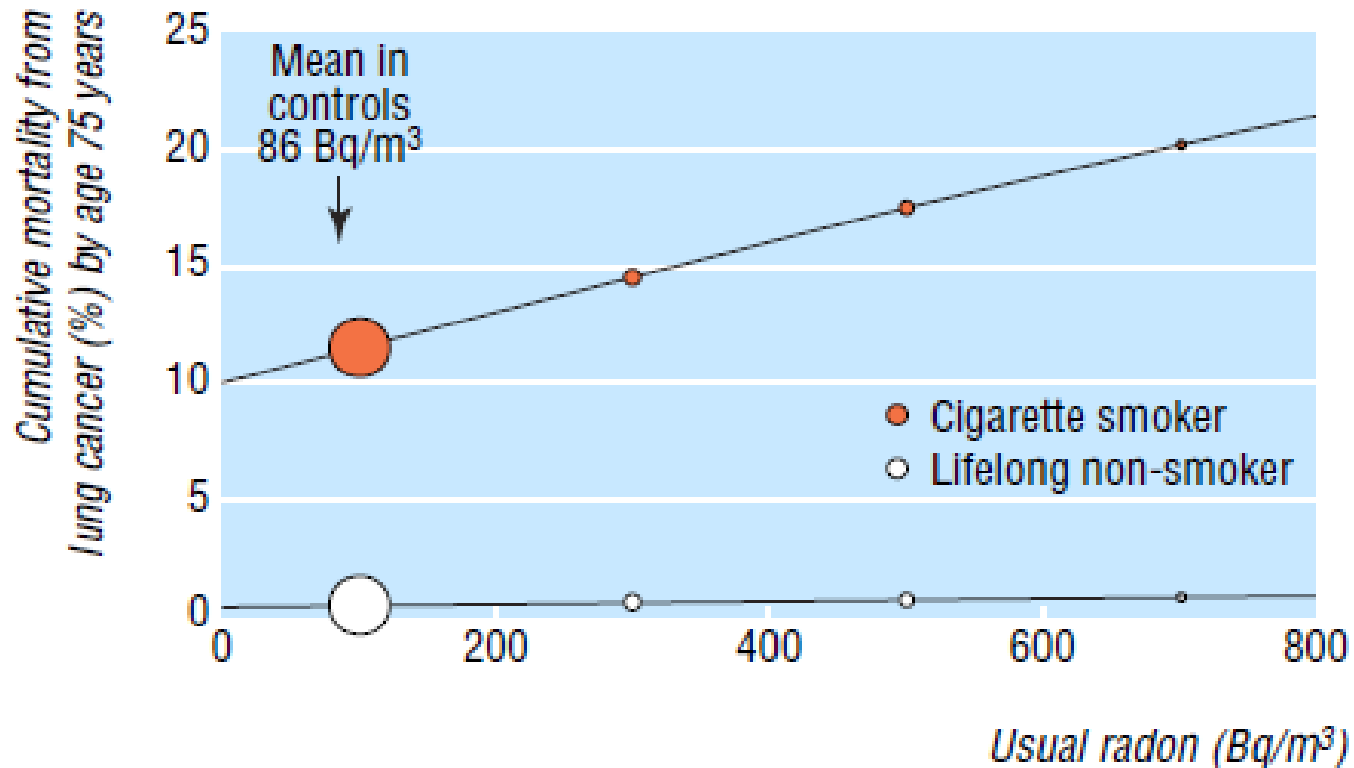
Darby et al. (2005), Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies. BMJ 330: 223-226
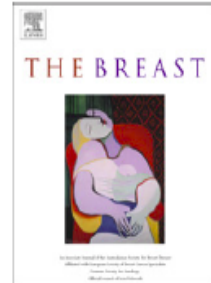
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Social-Genetic Interaction

Original article

Alcohol consumption and the risk of breast cancer among *BRCA1* and *BRCA2* mutation carriers

Jessica Dennis [a,b], Parviz Ghadirian [b,c], Julian Little [a,b], Jan Lubinski [d], Jacek Gronwald [d], Charmaine Kim-Sing [e], William Foulkes [f], Pal Moller [g], Henry T. Lynch [h], Susan L. Neuhausen [i], Susan Domchek [j], Susan Armel [k], Claudine Isaacs [l], Nadine Tung [m], Kevin Sweet [n], Peter Ainsworth [o], Ping Sun [p], Daniel Krewski [a,b], Steven Narod [p,*] the Hereditary Breast Cancer Clinical Study Group [q]
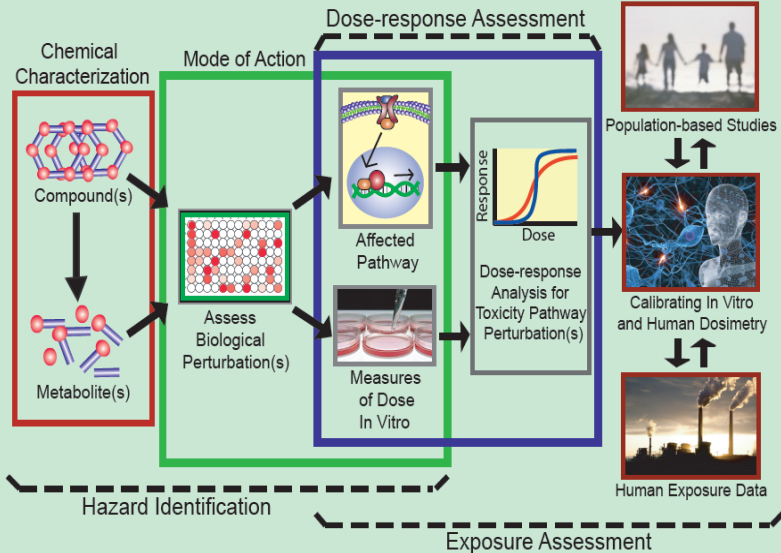
Risk
Management

Risk Management Principles (1/2)

- Beneficence and non-maleficence *(do more good than harm)*

- Natural justice *(a fair process of decision making)*

- Equity *(ensure an equitable distribution of risk)*

- Utility *(seek optimal use of limited risk management resources)*

- Honesty *(be clear on what can and cannot be done to reduce risk)*

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Risk Management Principles

- Acceptability of risk *(do not impose risks that are unacceptable to society)*

- Precaution *(be cautious in the face of uncertainty)*

- Autonomy *(foster informed risk decision-making for all stakeholders)*

- Flexibility *(continually adapt to new knowledge and understanding)*

- Practicality *(the complete elimination of risk is not possible)*
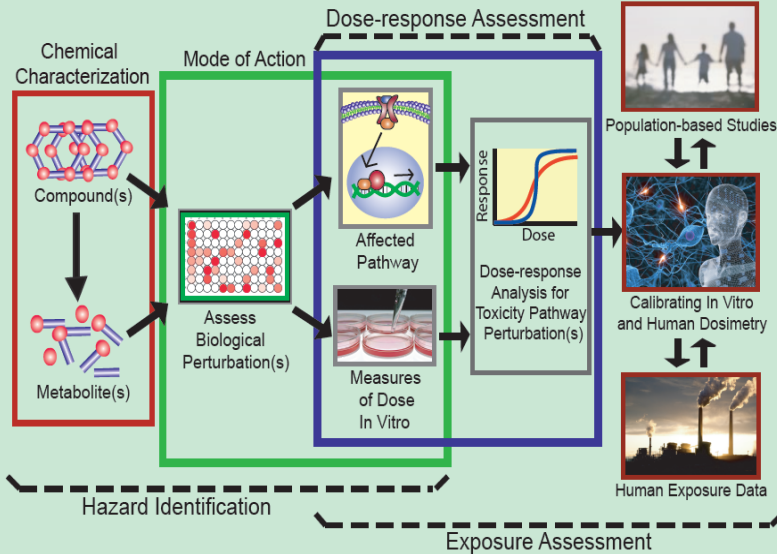
Case Study Prototypes

Tier 1 Assessments
- Screening and prioritization (thousands of chemicals)
- High throughput and QSAR driven
- Virtual tissue models (under development)
- Surrogates for or modeling of exposure/dose
- Minimize false negatives

Tier 2 Assessments
- More in depth evaluation of many chemicals (hundreds)
- Tier 1 approaches plus:
  - High-content assays
  - Some short-duration in vivo exposures
  - Expanded modeling of exposure/dose
  - Limited traditional data
- Science-based defaults and upper confidence limits

Tier 3 Assessments
- Extensive evaluation of few chemicals (dozens) – only highest hazard and exposure chemicals
- All feasible, policy-relevant, emerging and traditional data
- Omics supported epidemiology
- Best estimates of risk and uncertainty analyses

Research

New Test Data

Decision-making

LEGEND:
→ Decision-making/Policy Input
→ Decision-making-Testing - Research Loop
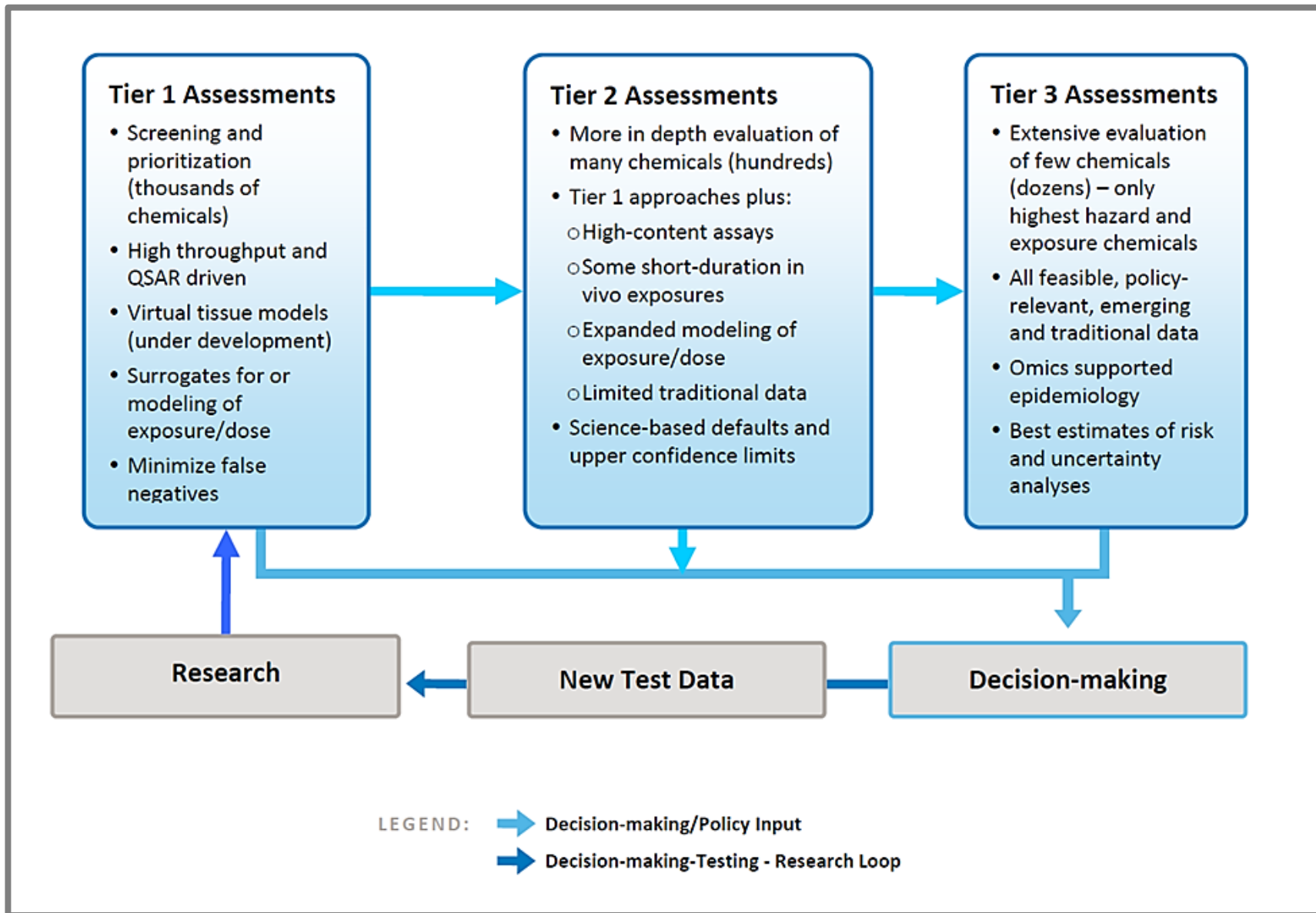
NexGen Tiered Approach to Risk Assessment
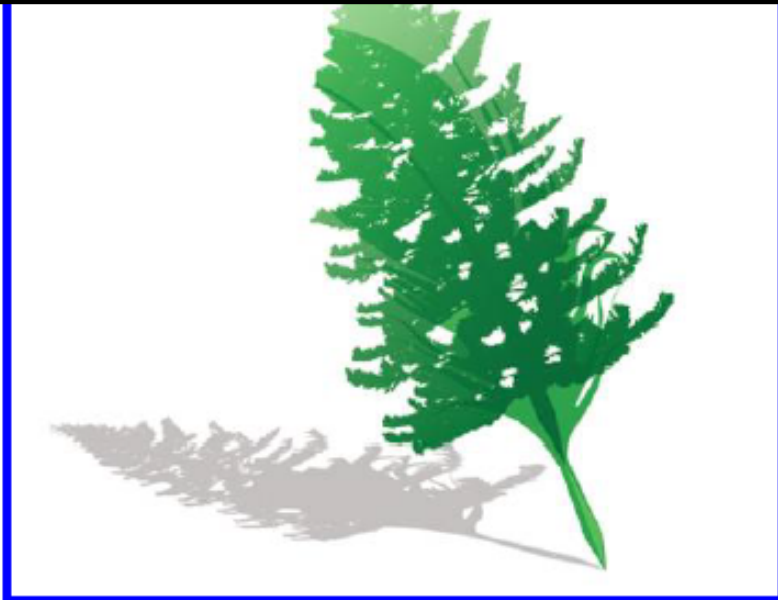www.epa.gov/risk/nexgen/docs/NexGen-Program-Synopsis.pdf

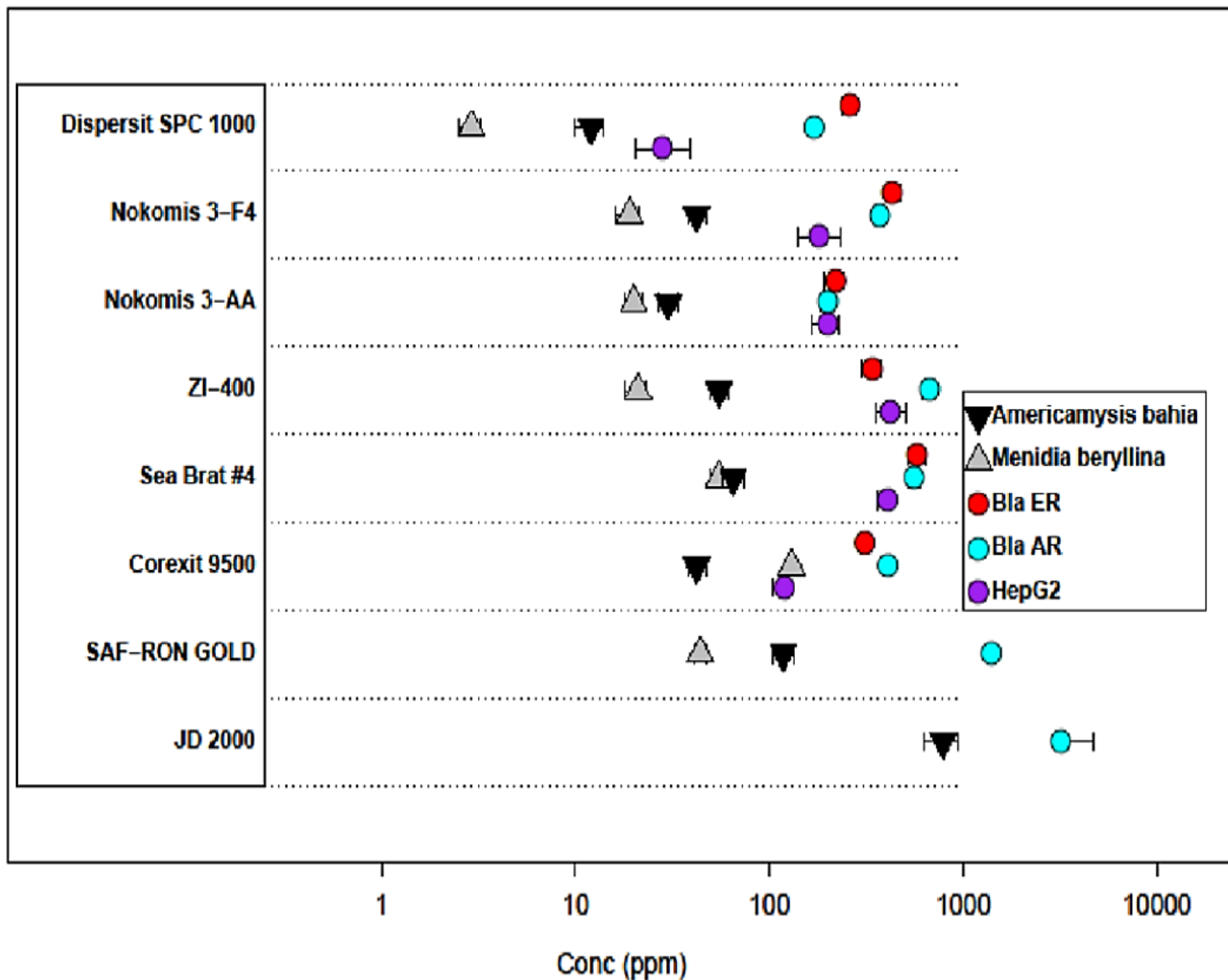**Designing Science in a Crisis: The Deepwater Horizon Oil Spill**

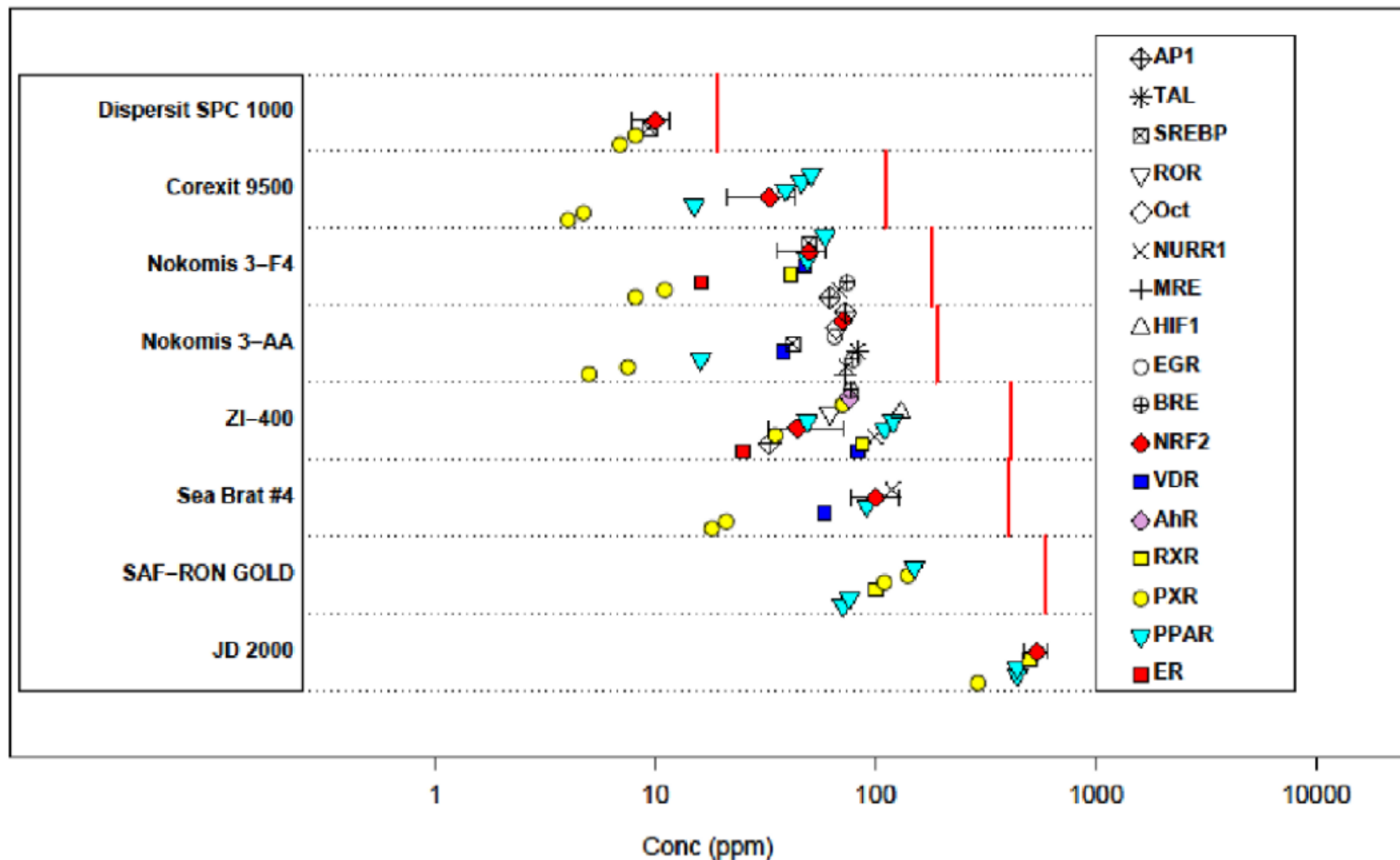PAUL T. ANASTAS*
CYNTHIA SONICH-MULLIN*
BECKY FRIED

Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Tier 1: Screening and Ranking

Judson et al. (2010), EST

Summary plot of results for all Attagene assays and the dispersants. Each horizontal band displays EC50 values for a single dispersant. Points are staggered in the y-direction to make overlapping points visible. Multiple assays for a given gene target (e.g. PPARα, PPARδ, PPARγ) are represented by a single symbol, plotted repeatedly. 95% confidence intervals are shown on assays for the NRF2 as an example. The dispersant-specific vertical red lines indicate the LC50 for cytotoxicity in the Attagene assays (HepG2 cells).

Tier 2: Limited Scope Assessment

Integrating pathway-based transcriptomic data into quantitative chemical risk assessment: A five chemical case study

Russell S. Thomas [a,*], Harvey J. Clewell III [a], Bruce C. Allen [b], Longlong Yang [a], Eric Healy [a], Melvin E. Andersen [a]

[a] The Hamner Institutes for Health Sciences, 6 Davis Drive, Research Triangle Park, NC 27709, United States
[b] Bruce Allen Consulting, 101 Corbin Hill Circle, Chapel Hill, NC 27514, United States
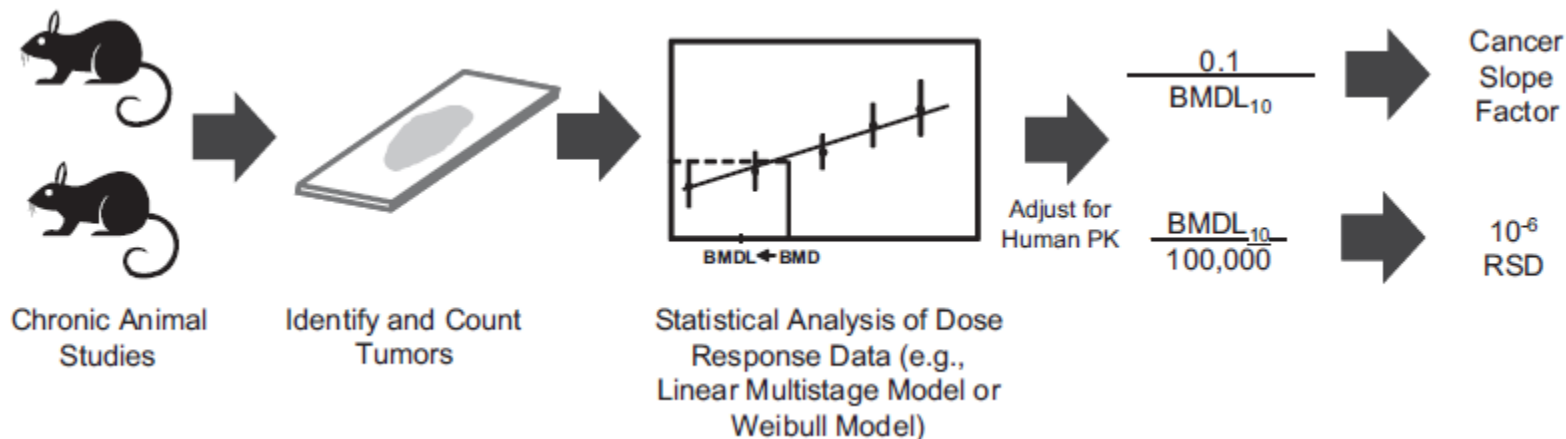
Traditional versus Toxicogenomics Determination of BMD

Transcriptional benchmark dose estimates for the most sensitive pathway.

Chemical[a]	GeneGo pathway map	Total genes in category/genes with BMD	Median BMD (mg/kg-d or mg/m^3)[b]	Median BMDL (mg/kg-d or mg/m^3)[b]
DCBZ	Neolacto-series GSL metabolism p.2 (ID: 905)	17/6	90.7	61.2
PGBE	Beta-alanine metabolism (ID:2313)	10/5	630.0	368.9
TCPN	Galactose metabolism (ID: 821)	21/6	9.5	4.5
MECL (liver)	CFTR translational fidelity (class I mutations) (ID: 2939)	77/59	1460.5	945.9
MECL (lung)	Folic acid metabolism (ID: 879)	14/5	1074.0	659.8
NPTH	Acetaminophen metabolism (ID: 2377)[c]	14/9	7.5	5.4

[a] DCBZ,1,4-dichlorobenzene; PGBE, propylene glycol mono-t-butyl ether; TCPN, 1,2,3-trichloropropane; MECL, methylene chloride; NPTH, naphthalene.
[b] Median transcriptional BMD and BMDL values for the associated GeneGo pathway map.
[c] GeneGo pathway map for acetaminophen metabolism contains five genes associated with Ugt1a isoforms that map to the same probe sets which skews the median value. This is due to the mouse Ugt1 locus that produces nine different genes through the alternative splicing of 14 variable exons to four constant exons [62].
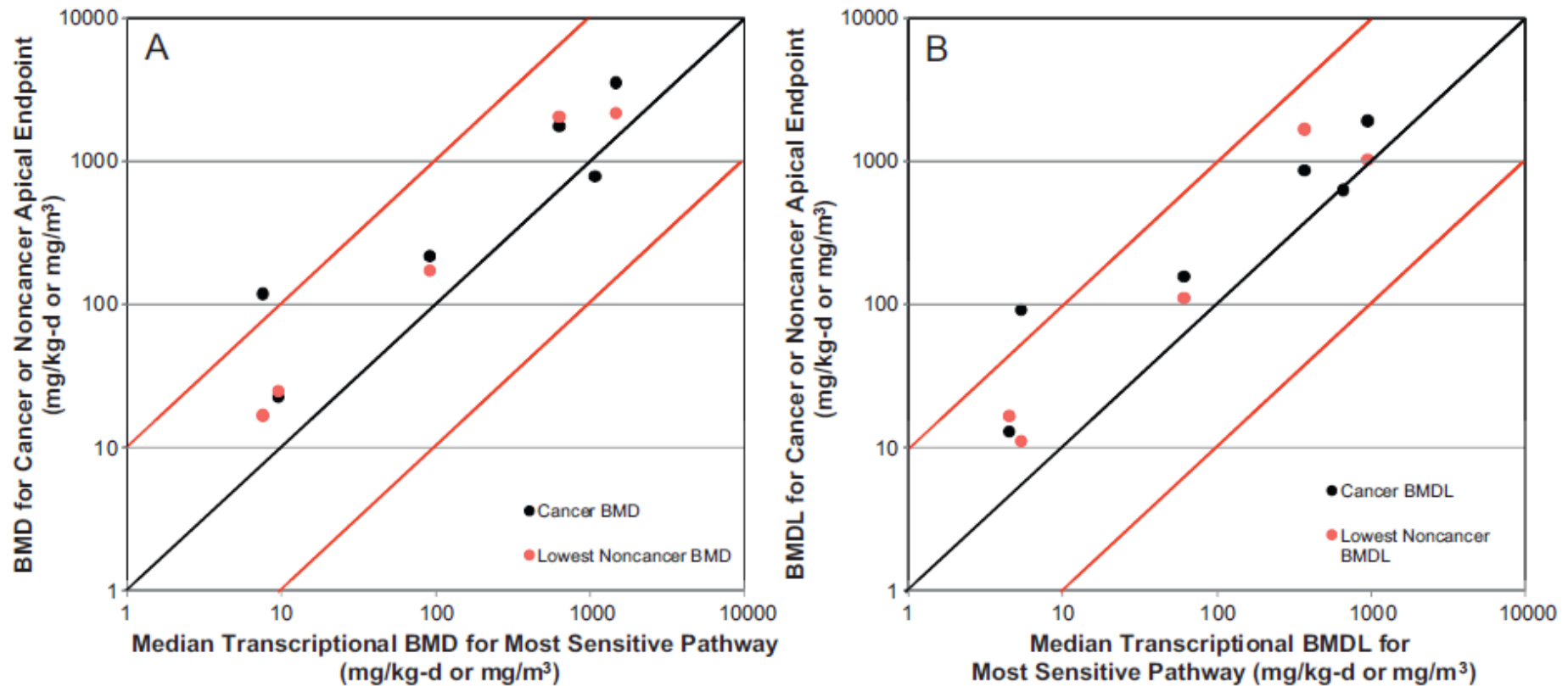
Thomas et al. (2012), Mutation Research

Fig. 5. Scatter plot of the relationship between (A) BMD and (B) BMDL values for the cancer and non-cancer apical endpoints and the transcriptional BMD and BMDL values for the most sensitive pathway. For each chemical and tissue, the BMD and BMDL values for tumor incidence and the lowest non-cancer BMD and BMDL values were plotted. For MECL in the lung, no non-cancer BMD or BMDL values were plotted due to the absence of histological changes. The red lines signify a 10-fold difference between the apical and transcriptional responses.

Thomas et al. (2012) found a strong correlation between transcriptional BMDs for specific pathways and traditional BMDs

Tier 3: Major Assessment

Lung Injury and Ozone

- To identify toxicity pathways using 'omics' data
- To determine accuracy of predicting in vivo responses from in vitro toxicity pathway induction from toxicants
- To develop a biologically-based dose-response (BBDR) based on the integration of diverse data sets

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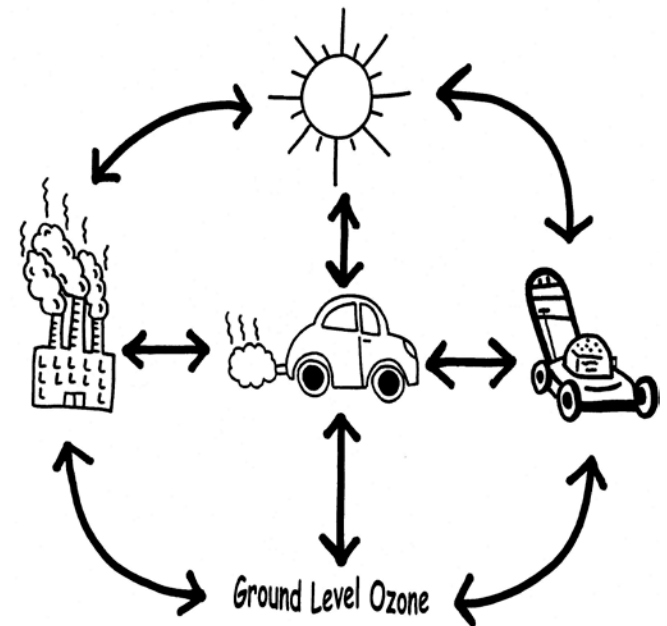
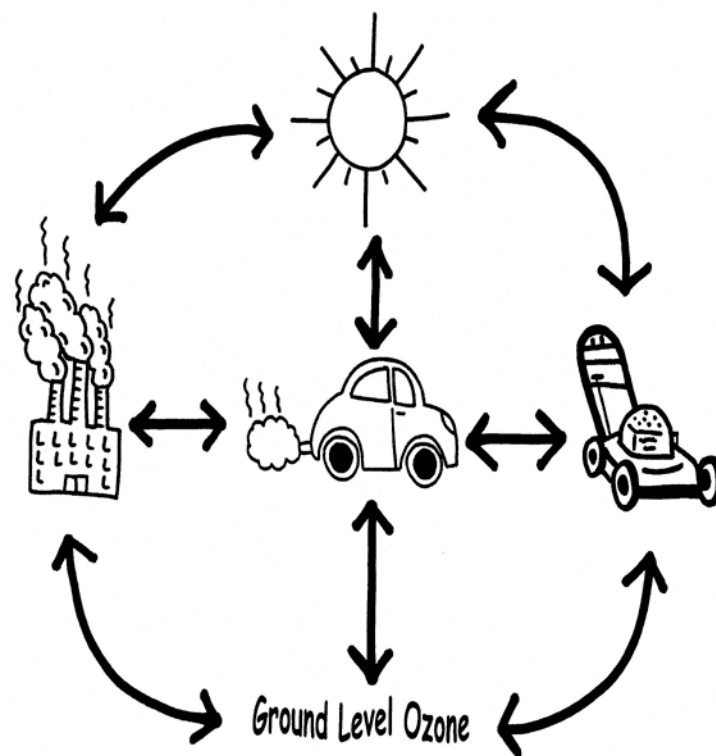**IL8 RNA Expression Time Course Relative to Mean Air Value**
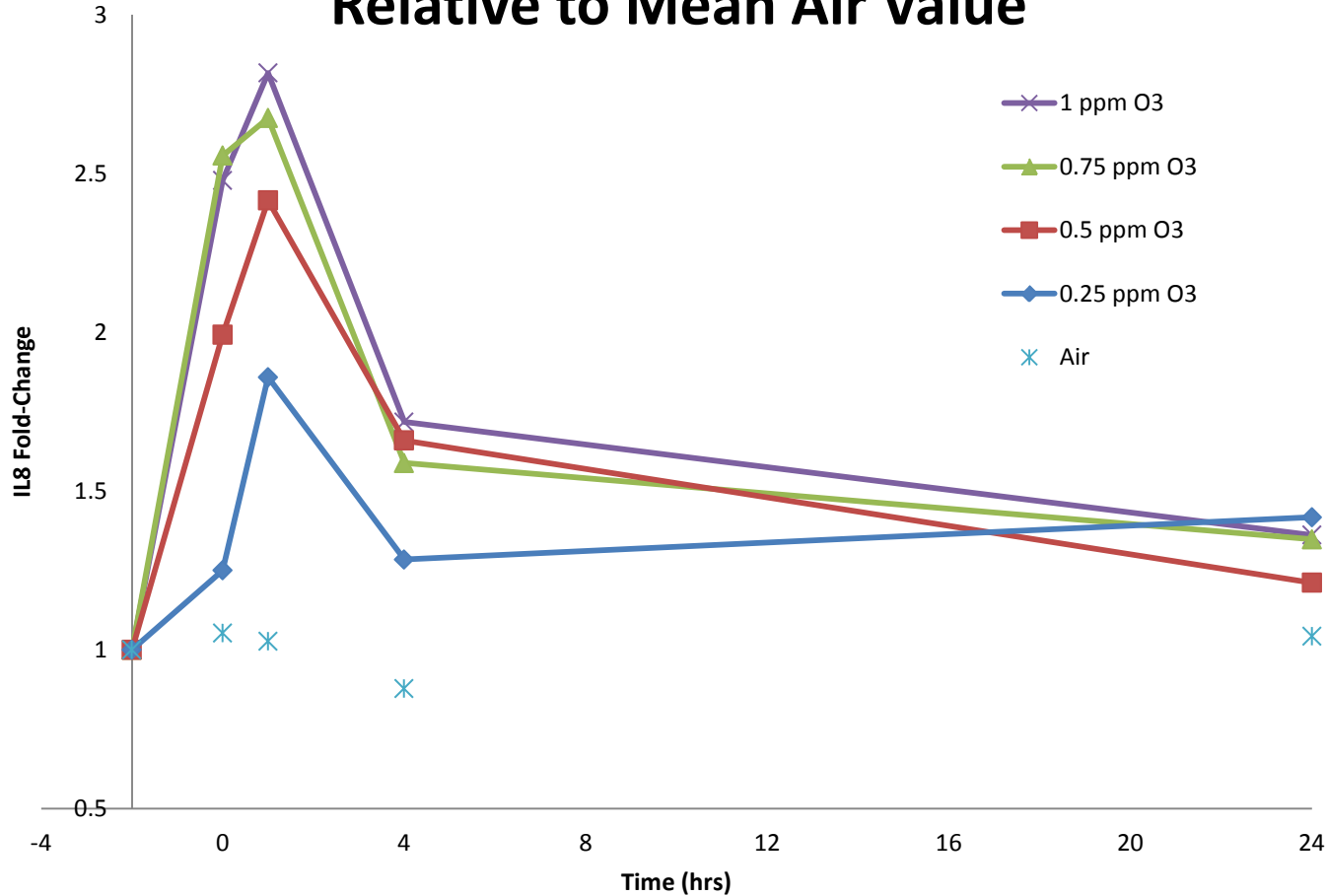
Response time course of IL8 RNA expression, relative to mean air value. Peak response occurs 3 hours post exposure cessation.
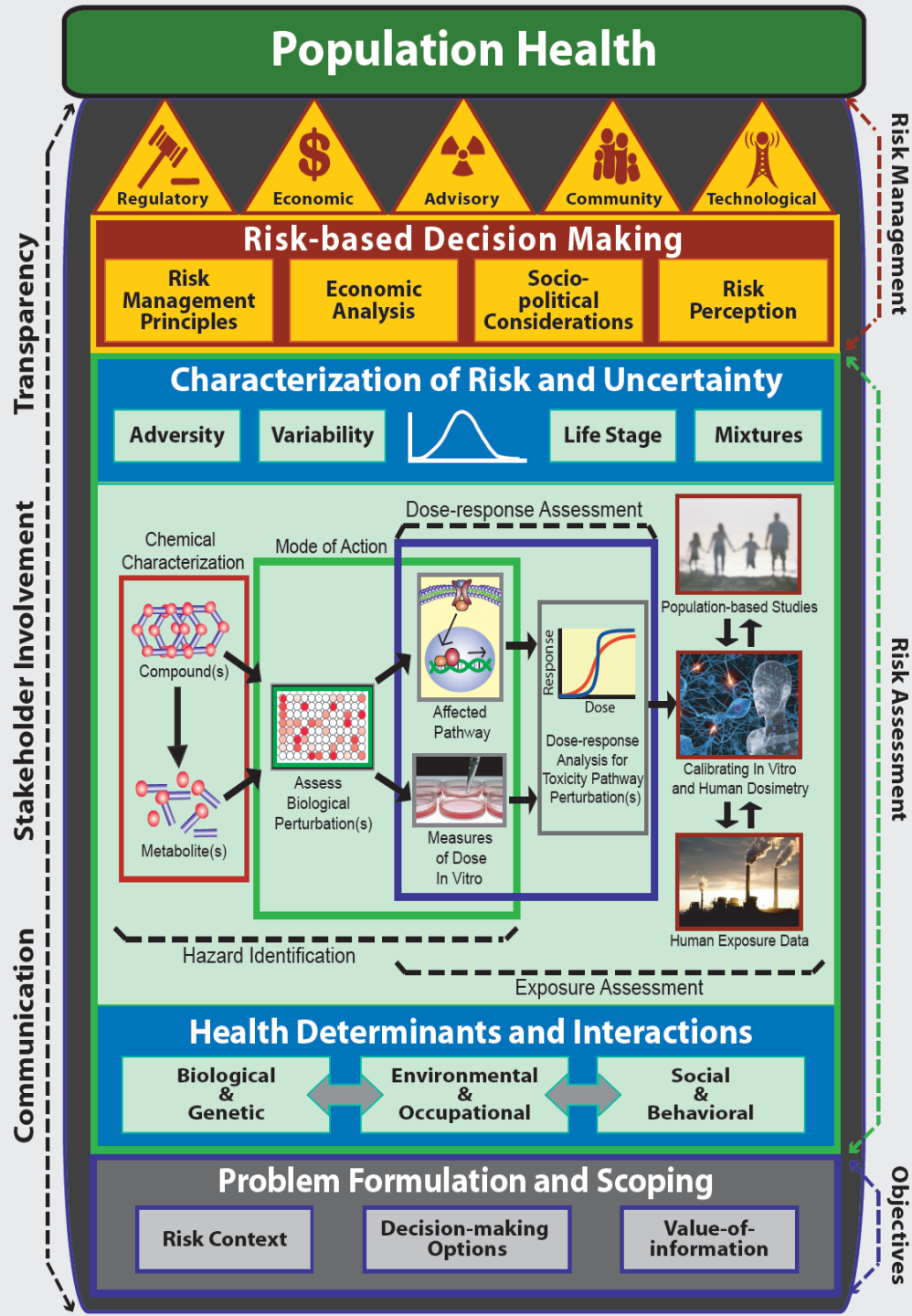
Lung Injury and Ozone

Conclusion

NF-κB signaling is seen early
during ozone exposure,
and there is a clear dose-response
with the cytokine IL-8.

Scientific Tools	Tier 1: Ranking and Screening		Tier 2: Limited Scope Assessment	Tier 3: Major Assessment	
	DWH Oil Spill Dispersants	Type-2 Diabetes	Short-term In Vivo Assay	Ozone and Lung Injury	Benzene and Cancer
Structure-activity relationships	√		√	√	√
MOA based in vitro toxicity pathway assays	√	√	√	√	√
High throughput screening	√		√	√	√
Stem cell biology					√
Functional genomics	√	√	√	√	√
Bioinformatics	√	√	√	√	√
Systems biology	√	√	√	√	√
Computational systems biology	√		√	√	√
PBPK and dosimetry			√	√	√
Toxicity-related biological pathway altering dose	√		√	√	√
Molecular and genetic epidemiology		√		√	√
Biomarkers	√	√	√	√	√

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