DISTINGUISHING ADVERSE FROM NON-ADVERSE/ADAPTIVE EFFECTS PROJECT COMMITTEE

Mission
The mission of the Project Committee on Distinguishing Adverse from Non-Adverse/Adaptive Effects is to develop an approach for the evaluation of the continuum of effects observed in toxicological investigations ranging from benign to adverse, and to use this approach to facilitate the integration and utilization of biological information in the safety assessment of chemicals/pharmaceuticals

2012-2013 Participating Organizations
BASF Corporation
Brown University
Colorado State University
Dow AgroSciences
ExxonMobil Biomedical Sciences, Inc.
Monsanto Company
National Institute of Environmental Health Sciences
Sanofi
US Environmental Protection Agency

Committee Publications
Keller, D. A., Juberg, D. R., Catlin, N., Farland, W. H., Hess, F. G., Wolf, D. C., and Doerrer, N. G. (2012). Identification and characterization of adverse effects in 21st century toxicology. Toxicol Sci 126(2), 291-7. More details

2012-2013 Activities and Accomplishments

Committee leaders:
Dr. William Farland
Colorado State University

Dr. Daland Juberg
Dow AgroSciences

Dr. Douglas Keller
Sanofi

HESI manager:
Nancy G. Doerrer, MS

This scientific program is committed to:
Developing an approach for the evaluation of the continuum of effects observed in toxicological investigations ranging from benign to adverse, and applying this approach to facilitate the integration and utilization of biological information in the safety assessment of chemicals and pharmaceuticals.

Areas of scientific focus:

- Explore how information from new, high data content assays developed for screening can be used to differentiate adverse effects from adaptive responses.
- Develop criteria for determining whether an effect is potentially adverse or adaptive, and examine data for prototypical, data-rich compounds as a means to gaining greater understanding of relevant pathways of toxicological concern.
- Catalyze dialogue and research on characterizing relevant pathways of toxicological concern and their use in risk assessment and public health protection.

Why get involved?
The project committee was sunset in December 2012.

Key accomplishments:

- *Workshop Publication.* A Forum paper was published in *Toxicological Sciences* in 2012 as a result of the project committee's May 2011 workshop on "Distinguishing Adverse from Adaptive Effects in the 21st Century," held at the US Environmental Protection Agency (EPA) facilities in Research Triangle Park, North Carolina. The paper provides an overview of key issues discussed prior to and during the workshop, including the use of data and high data content information from *in vitro* studies to inform decisions about adversity and the application of such information in a risk assessment context.
- *Outreach.* In June 2012, the project committee's work was featured during a webinar sponsored by the Society of Toxicology (SOT) Risk Assessment Specialty Section. The presentation, titled "Identification and Characterization of Adverse Effects in 21st Century Toxicology and Risk Assessment," was well received by the >90 attendees.

The Committee's focus for May 2013 - May 2014:
SOT Workshop. During the past year, the HESI Project Committee leadership worked closely with scientists from the US EPA National Center for Computational Toxicology and others to develop a joint SOT Contemporary Concepts in Toxicology (CCT) workshop proposal that includes a focus on determining the extent to which pathway-level perturbations reflect an adverse (toxicological) consequence versus an adaptive (compensatory) response. The SOT-approved workshop, titled "FutureTox II: *In Vitro* Data and *In Silico* Models for Predictive Toxicology," will be held January 16–17, 2014, at the William and Ida Friday Center for Continuing Education at the University of North Carolina in Research Triangle Park, North Carolina.

Although the HESI Project Committee is sunset, the committee leadership and staff are represented on the SOT Workshop Organizing Committee. HESI is a co-sponsor of the event.

Providing support for the SOT CCT workshop beyond the life of the HESI committee was important to committee participants. This opportunity will encourage the use of rigorous, standardized *in vitro* and/or *in silico* data to predict later-occurring apical endpoints from precursor dose transitions in relevant pathways of toxicological concern.

Recent publications:
Keller DA, Juberg DR, Catlin N, Farland WH, Hess FG, Wolf DC, Doerrer NG. (2012). Identification and characterization of adverse effects in 21st century toxicology. *Toxicol Sci.* 126(2): 291–297.

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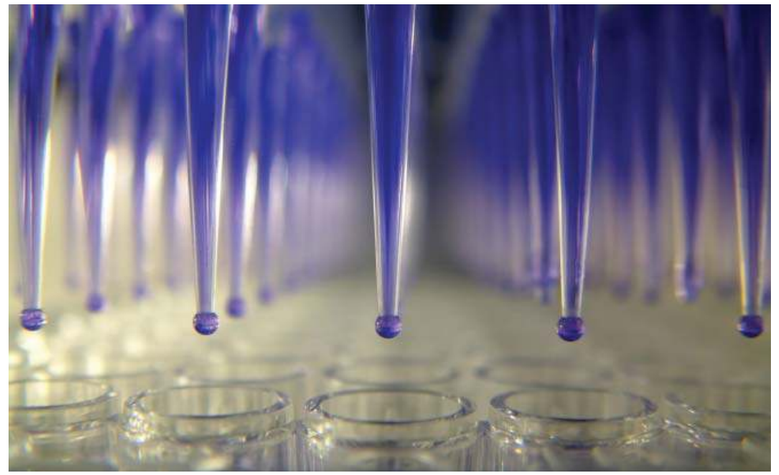
US Food and Drug Administration

For more information, contact the Committee's manager, Ms. Nancy G. Doerrer, ndoerrer@hesiglobal.org.



Committee Presentations and Data Resources

May 13, 2010: Presentation. "Subcommittee on Distinguishing Adverse from Non-Adverse and Adaptive Effects." Presentation by Dr. Douglas A. Keller (sanofi aventis, US) on behalf of the HESI Subcommittee on Distinguising Adverser fron Non-Adverse/Adaptive Effects. HESI 2010 Annual Meeting, Reston, VA.

H E S I®

Subcommittee on Distinguishing Adverse from Non-Adverse and Adaptive Effects

DOUGLAS A. KELLER, PhD
(sanofi-aventis US)
Subcommittee Co-Chair

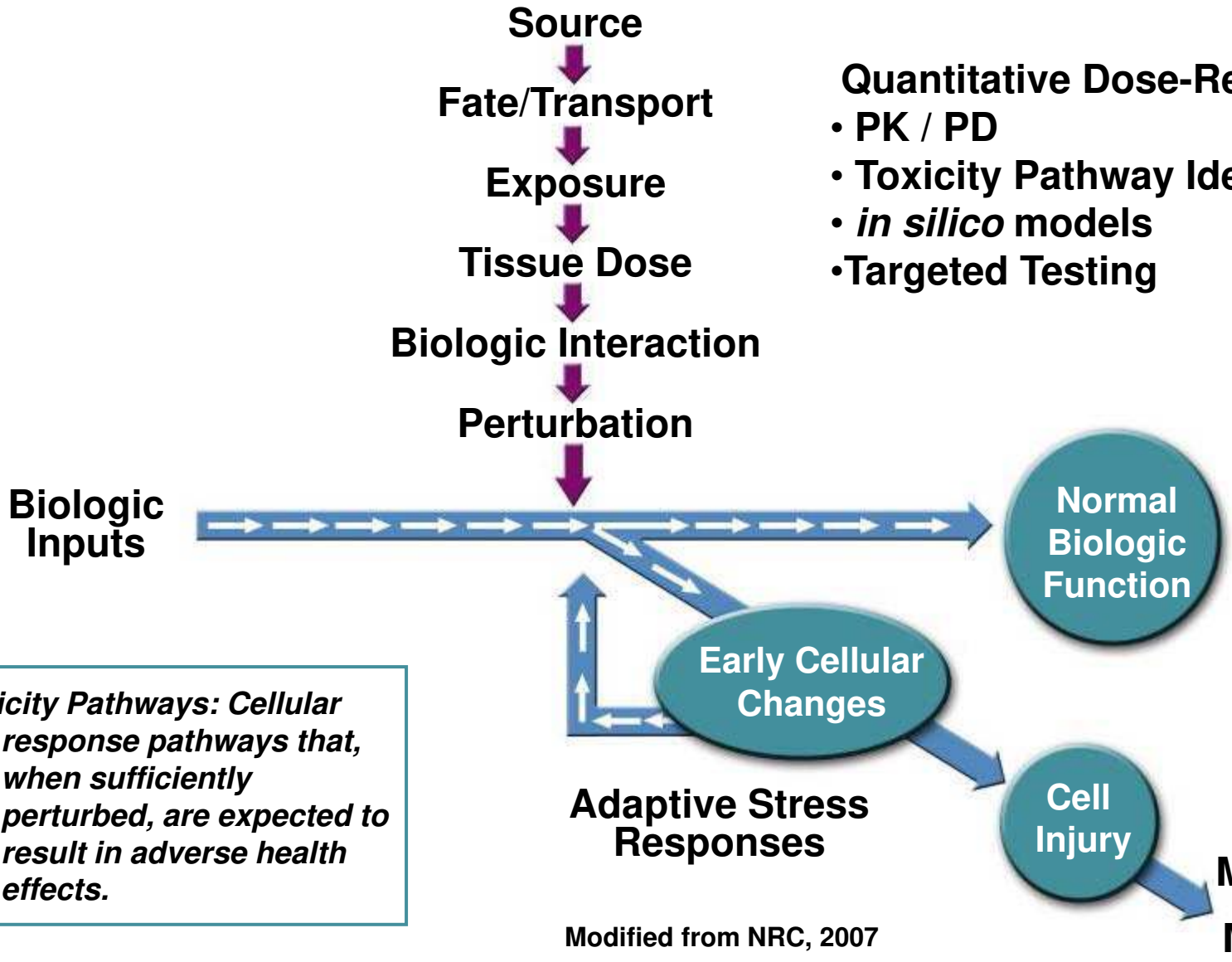
HESI Annual Meeting
Reston, VA
May 13, 2010

The issue

- Advances in technology are changing approaches to toxicology testing
  - Molecular mechanisms
  - Biomarkers
  - 'Omics
- The NRC has suggested a new vision and strategy for toxicology testing
  - Shift from whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin

The data from these studies do not fit neatly into the analysis paradigm of "adverse" or "not adverse" developed over many years from in vivo studies

Applying a Systems Toxicology Approach



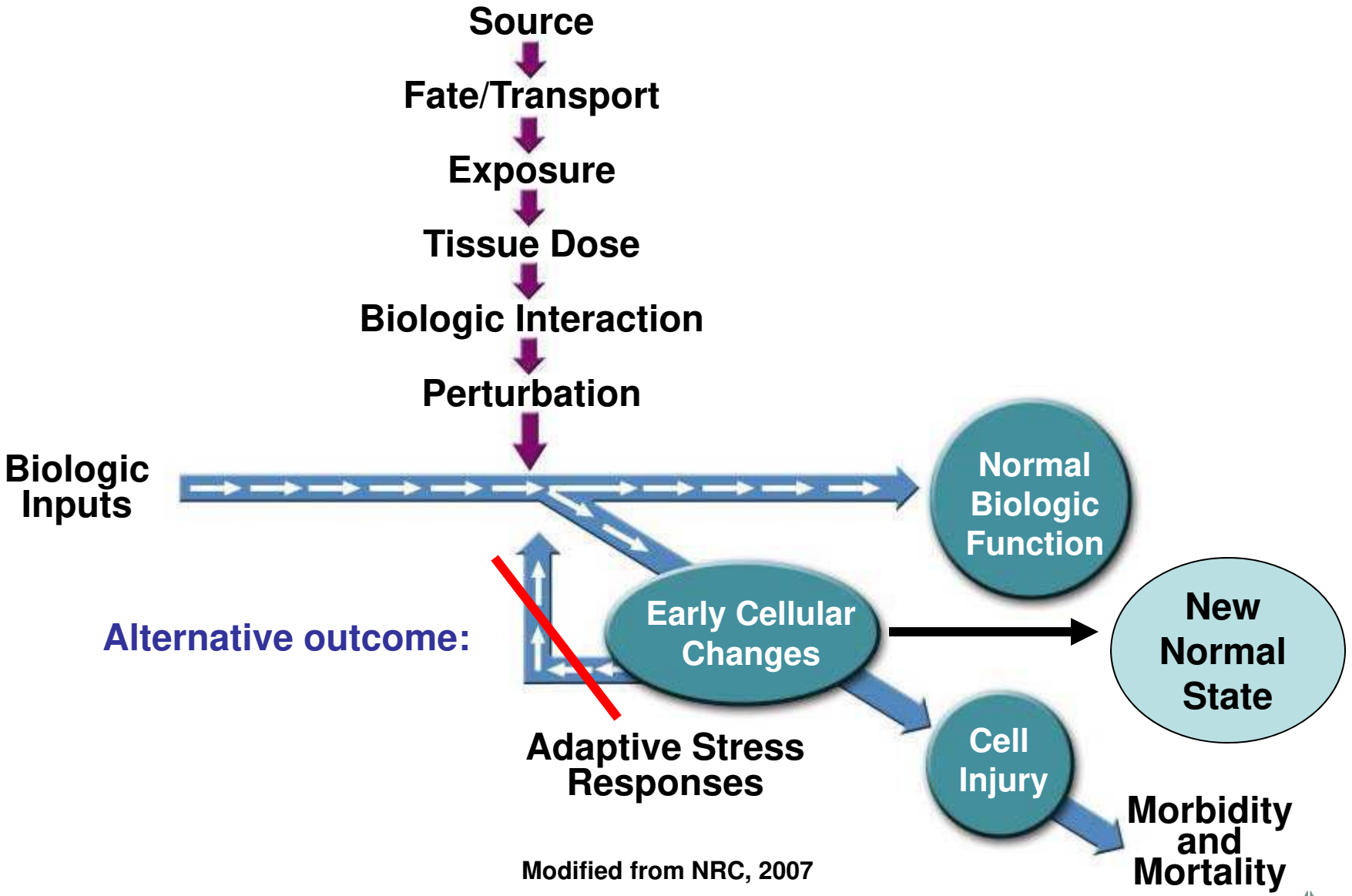
Quantitative Dose-Response

- **PK / PD**
- **Toxicity Pathway Identification**
- ***in silico* models**
- **Targeted Testing**

Toxicity Pathways: Cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects.

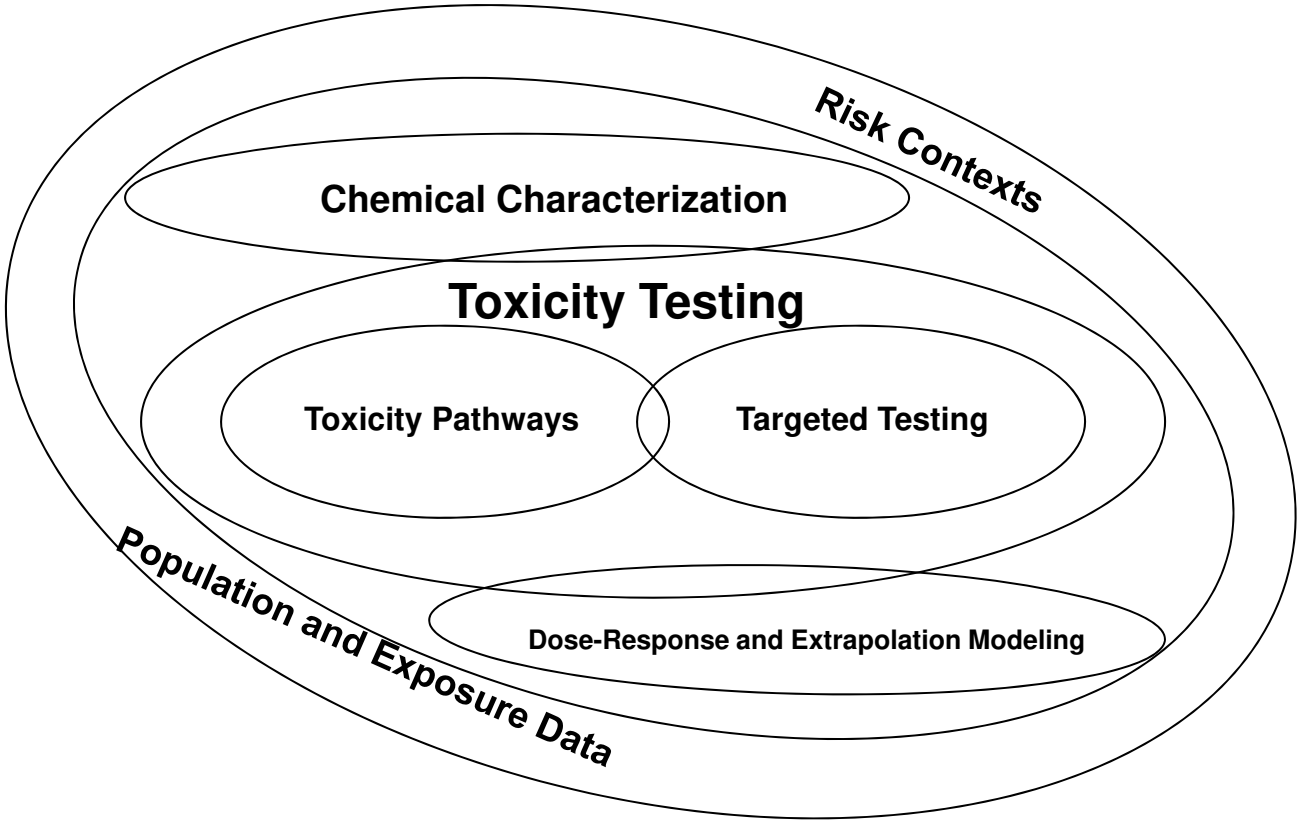
Modified from NRC, 2007

Applying a Systems Toxicology Approach



Modified from NRC, 2007

NRC's 21st Century Vision for Toxicity Testing



Adapted from NRC (2007)

Why is this issue important?

- In the absence of compelling human data (rarely available), doses that cause adverse effects in animals are used to regulate:
  - Allowable concentrations in air, water, soil, crops
  - Doses used in clinical trials of drugs
  - Exposure limits in occupational settings
- Setting an adverse effect level lower than scientifically justified can have a high economic impact
  - Expensive emission controls
  - Protracted, expensive remediation
  - Longer, more expensive pharmaceutical development
  - Discontinued development of potentially useful chemicals and pharmaceuticals
  - Etc.
- Setting an adverse effect level higher than scientifically justified can lead to unwarranted risk
- The use of mechanistic and molecular information in risk assessment is not well defined

Subcommittee accomplishments of the first year

- Organizational teleconference held December 2008
- First face-to-face meeting held March 19, 2009
  - Agreed on mission and objectives
  - Began compiling background materials, relevant literature and consider case studies
- April 2009
  - Subcommittee website developed
  - List of reference terms compiled for use in developing framework
- July 2009
  - Began developing criteria for evaluating adverse vs. adaptive effects
  - Agreed to develop strawman definitions for “adverse” and “adaptive”
  - Case study concept further developed

Subcommittee accomplishments of the first year

- September 2009 (2nd face-to-face meeting)
  - Agreed on draft definitions of “adverse” and “adaptive” effects
  - Draft framework developed
  - First case study selected
- October – December 2009
  - Evaluation of data sets for acetaminophen
  - Revision of framework questions
  - Connections established with NIEHS
- January – February 2010
  - Revision of framework questions
  - Draft flowchart developed
  - Matrix comparing framework categories developed
  - 2nd case study selected
  - Connections established with HESI Genomics committee
- March 2010 (3rd face-to-face meeting)
  - Expanded interactions with academic partners (K. Boekelheide)
  - Reviewed framework questions and flow chart with 2nd case study (dimethylarsinate)

Mission Statement

The mission of this subcommittee is to develop an approach for the evaluation of the continuum of effects observed in toxicological investigations ranging from benign to adverse, and to use this approach to facilitate the integration and utilization of biological information in the safety assessment of chemicals/pharmaceuticals.

Objective A

- Develop criteria to facilitate the determination of adverse from other types of changes (e.g., pharmacologic, adaptive, homeostatic, or non-functional). These criteria may include biologically relevant information such as temporality, genomic and tissue response, and identification of target organ or system.

Objective B

Develop an evaluation framework that integrates and prioritizes the information that characterizes an observed/measured change in a biological system. The framework will address the challenges in characterizing a change in the context of a continuum of effects (from benign to adverse) for which the characterization of a single effect may vary depending on the context. This framework will facilitate decision-making by providing clarity of information considered, their relative importance, and the risk context.

Definitions agreed on (but subject to development)

- ***Adverse Effect:*** **A change in morphology, physiology, growth, development, reproduction, or life span of a cell or organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.**

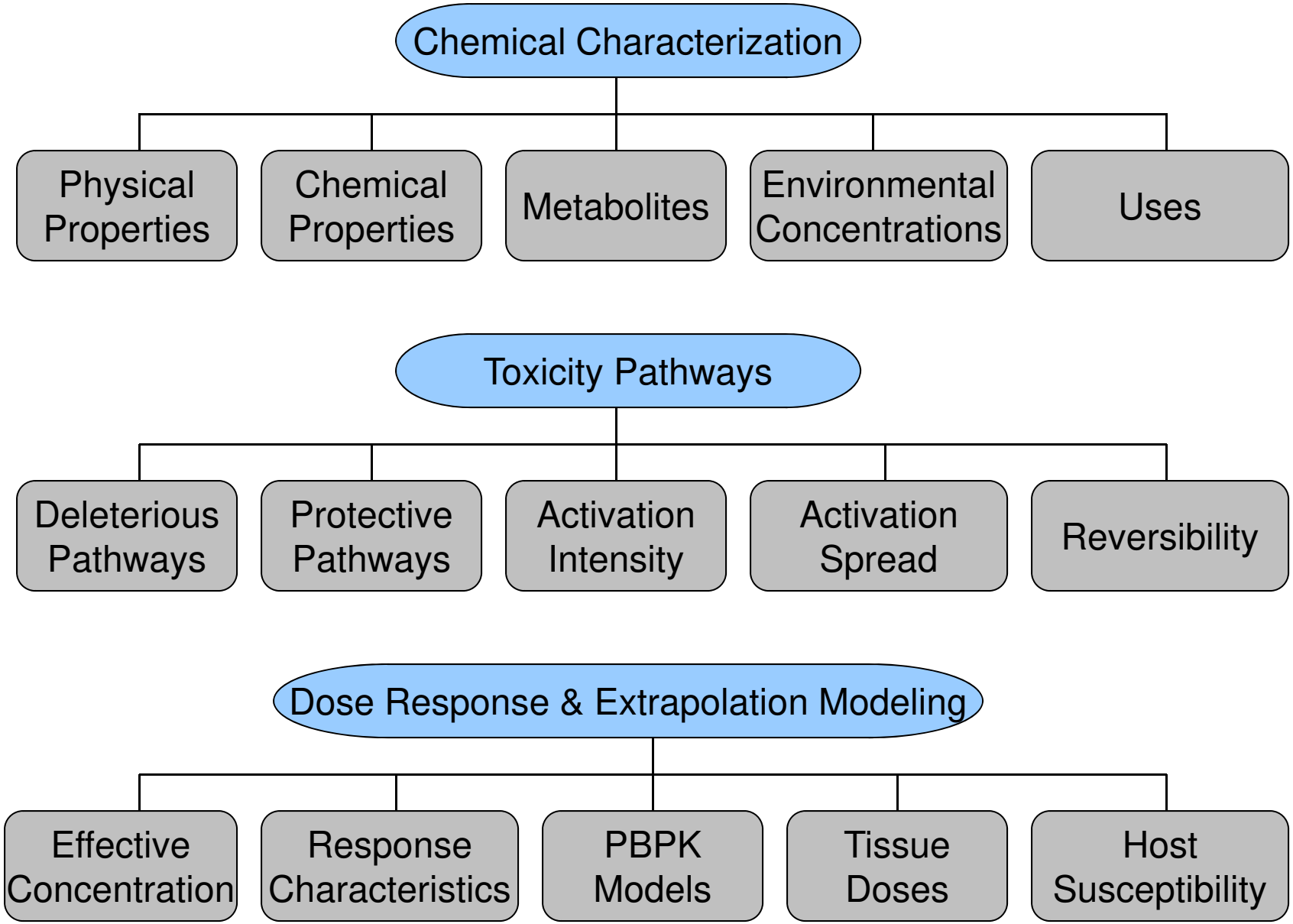
- ***Adaptive Effect:*** **In the context of toxicology, the process whereby a cell or organism responds to a xenobiotic so that the cell or organism will survive in the new environment that contains the xenobiotic without impairment of function.**

The framework – first pass

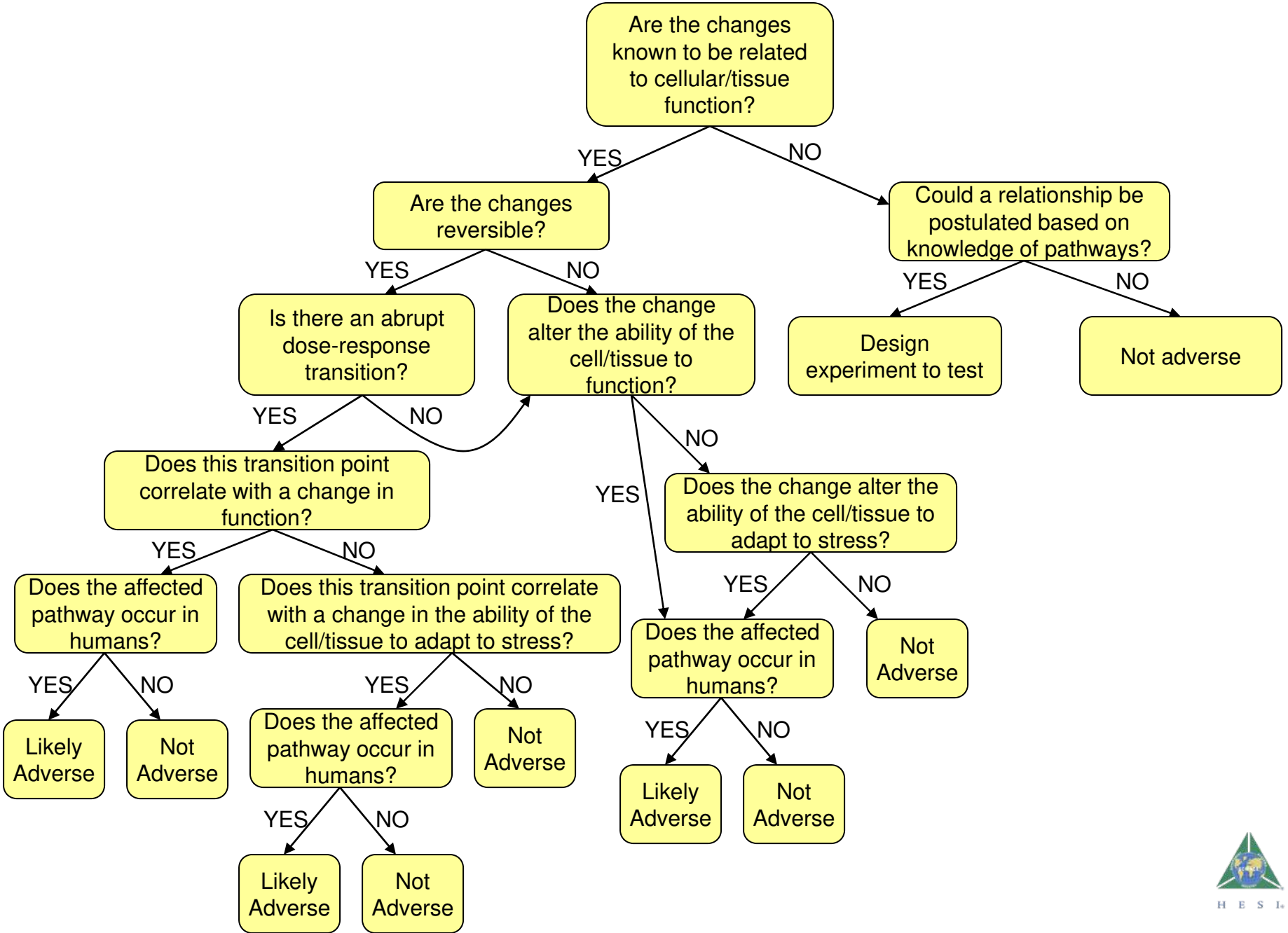
- **Characterization of the effect**
  - What is the observation? Reversible? Dose-response? Etc.
- **Relative placement of the effect to other levels of biological organization**
  - Key event in known or postulated MOA? Known to be associated with altered organ/tissue/system function? Depleted physiological reserve? Precursor to another effect? etc.
- **Human relevance**
  - Does or can the effect occur in humans? Relevance of dose levels to humans? MOA known in humans?

The TFACS Framework

Boekelheide and Campion, Toxicol. Sci. 2010 114: 20-24



Adverse vs. Adaptive DRAFT Flow Chart



Evaluation and refinement of the framework

Case studies being evaluated

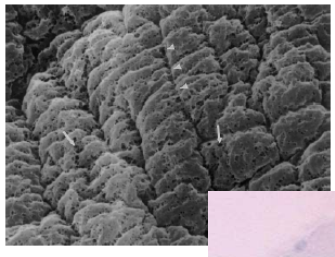
- **Acetaminophen hepatic toxicity**
  - Rich data set of histopathology, clinical chemistry, biochemical endpoints, toxicogenomics
  - Known human relevance
- **Dimethylarsenate urinary bladder carcinogenicity**
  - Rich data set of histopathology, in vitro cytotoxicity, biochemical endpoints, toxicogenomics
  - Established MOA

Dimethylarsenate: key events in mode of action

DMA^{V} → DMA^{III}

↓

(sustained) **Cytotoxicity**



Urinary bladder from a female F344 treated with 100 ppm DMA^{V}

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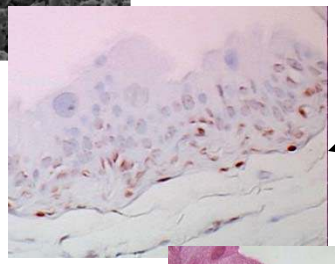

BrdU Labeling

(sustained) **Enhanced Cell Proliferation**

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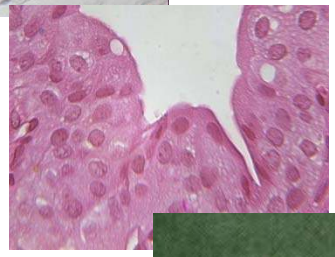

Hyperplasia

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Tumors

Urinary bladder tumors

Association of key precursor events & bladder tumors in F344 rats

Temporal

Dose Response Concordance

Dose (ppm)	Metabolism of DMA^{V} to DMA^{III}	Urothelial toxicity	Regenerative proliferation response	Urothelial hyperplasia	Transitional cell carcinoma
2	+ (week 3 0.03 ± 0.01 uM)	+ (week 10 6/10, grade 3 or 4)	-	-	-
10	+ (week 3 0.12 ± 0.02 uM)	+ (week 3 2/7, grade 3) (week 10 8/10, grade 3 or 4)	slight (week 10 1.5-fold increase)	-	-
40	+ (week 3 0.28 ± 0.09 uM)	+ (week 3 7/7, grade 3) (week 10 5/10, grade 3 or 4)	+ (week 10 4.3-fold increase)	+ (week 10 4/10)	-
100	+ (week 3 0.55 ± 0.15 uM)	+ (6 hrs 6/7, grade 3) (24 hrs 4/7, grade 3 or 4) (week 2 6/10, grade 5) (week 10 10/10, grade 4 or 5)	+ (week 1 2.2-fold increase) (week 2 3.9 fold) (week 10 4.2-fold increase)	+ (week 2 1/10) (week 8 7/10) (week 10 9/10)	+ (Gur et al., 1989; serial sacrifices not performed but papilloma first observed at week 107; carcinoma first observed at week 87)

Moving to in vitro toxicogenomic studies …

Table 1
Number of genes significantly altered across different dose groups in different model systems

Dose	Rat *in vivo*[d]	Rat *in vitro*[d]	Human in vitro[e]
40 ppm	124[a] (37[b]↑/16[c]↓)		
1 ppm	709[a] (302[b]↑/63[c]↓)		
0.1 ppm	144[a] (14[b]↑/52[c]↓)		
0.01 ppm	161[a] (26[b]↑/17[c]↓)		
8000 ppb		1701[a] (7[b]↑/238↓)	945[a] (201[b]↑/71[c]↓)
200 ppb		889[a] (14[b]↑/87↓)	1339[a] (249[b]↑/140[c]↓)
20 ppb		1943[a] (71[b]↑/402↓)	1668[a] (380[b]↑/227[c]↓)
2 ppb		917[a] (22[b]↑/183↓)	1436[a](322[b]↑/326[c]↓)

[a] Number of significant genes differentially expressed at each dose group compared to controls (Student *t*-test, $p < 0.05$) in the three model systems.
[b] Number of genes out of the total significantly altered genes that are up regulated as compared to controls.
[c] Number of genes out of the total significantly altered genes that are down regulated as compared to controls.
[d] Fold change cut off = ±1.5-fold.
[e] Fold change cut off = ±1.2-fold.

From: Sen et al., Toxicology in vitro 21 (2007), 1513-1529

Genes vs. pathways: How do we evaluate them?
(Will get input from HESI Genomics Technical Committee)

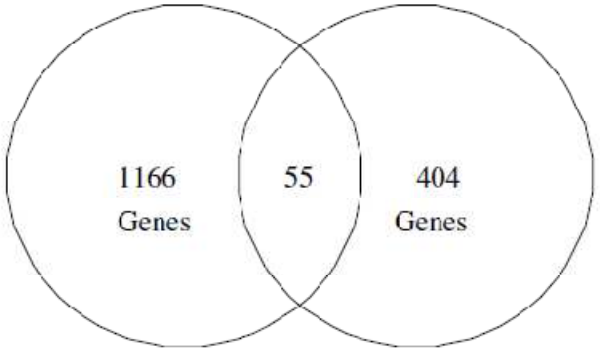


Fig. 2a. Venn analysis showing the common and unique number of significant genes across the *in vivo* and *in vitro* rat model systems.

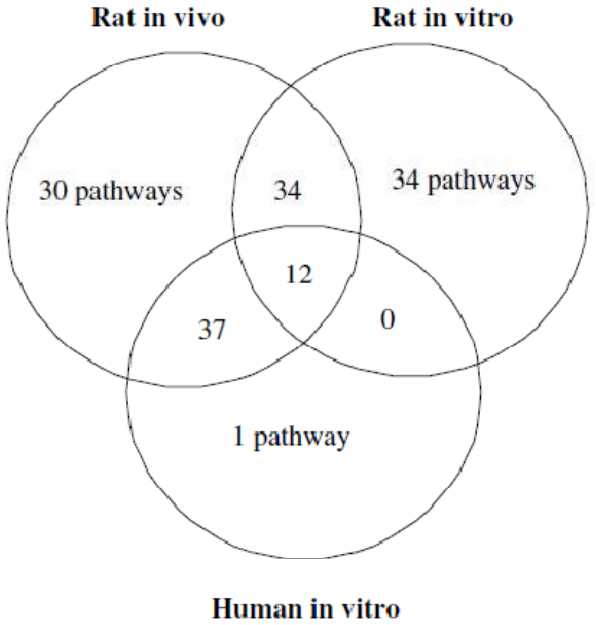
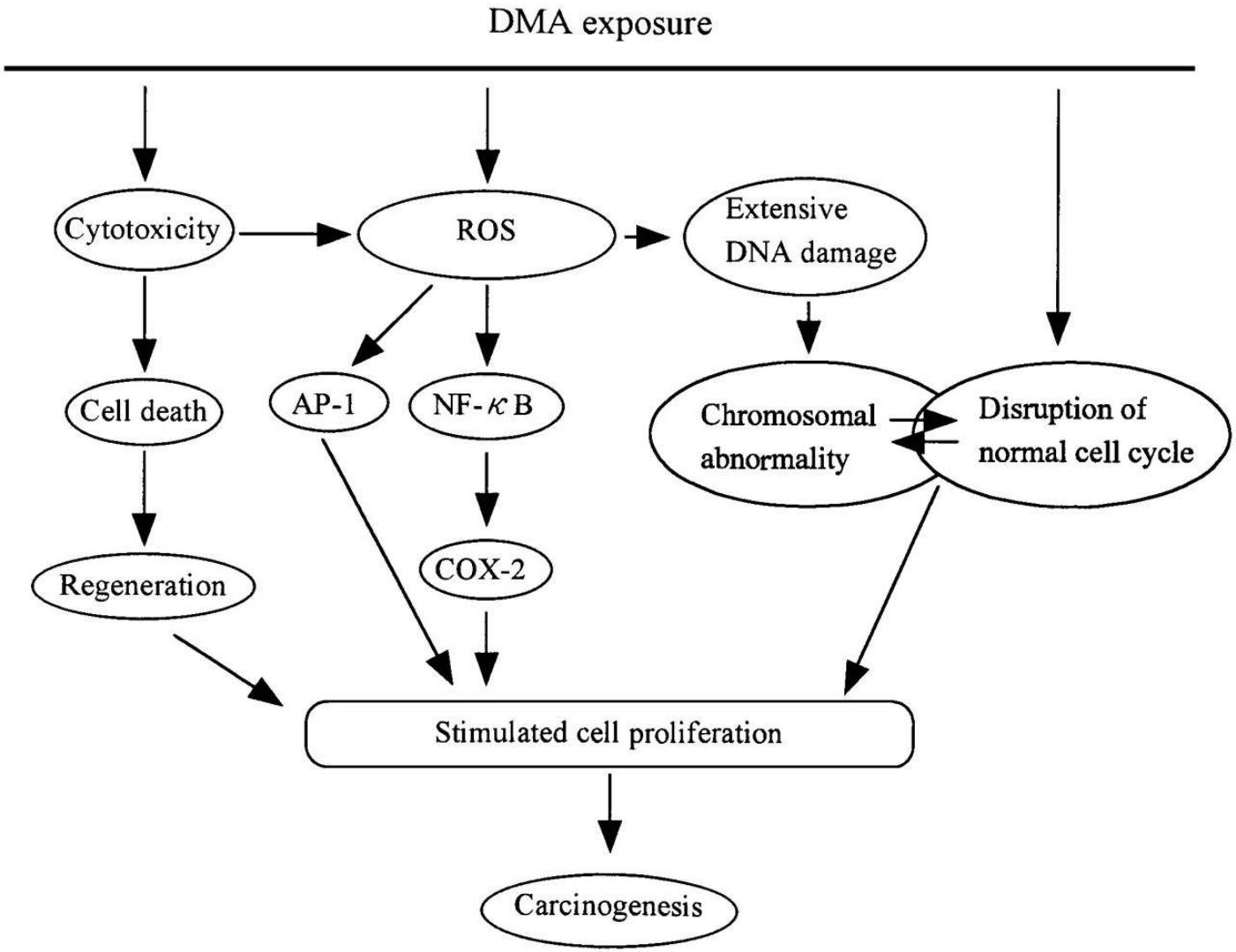


Fig. 2b. Venn analysis showing the number of common and unique significant pathways across *in vivo* and *in vitro* rat and *in vitro* human bladder model systems.

From: Sen et al., Toxicology in vitro 21 (2007), 1513-1529

Pathways of interest



From: Wei et al., Carcinogenesis 23, 1387-1397, 2002

Next steps

Goal is to

- Refine framework with these cases (within subcommittee)
- Get input from outside groups with small workshop in early to mid 2011
- Refine and test with new cases (2011)
- Potential for another workshop in the future
- Eventual publication of results

Subcommittee participants

- Doug Keller (Co-Chair), sanofi-aventis
- Daland Juberg (Co-chair) Dow AgroSciences
- Lauren Black, Charles River
- Kim Boekelheide, Brown University
- David Brewster, Hoffmann-LaRoche
- Albert DeFelice, US FDA
- Steven Durham, Charles River
- William Farland, Colorado State University
- Lee Geiger, GlaxoSmithKline
- Amber Goetz, Syngenta
- Frederick Hess, BASF
- Peter Mann, EPL
- Chris Portier, NIEHS
- David Saltmiras, Monsanto
- William Sette, US EPA
- Doug Wolf, US EPA
- HESI staff: Syril Pettit (2009), Nancy Doerrer (2010-), Cyndi Nobles