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Subcommittee on Distinguishing Adverse from Non-Adverse and Adaptive Effects

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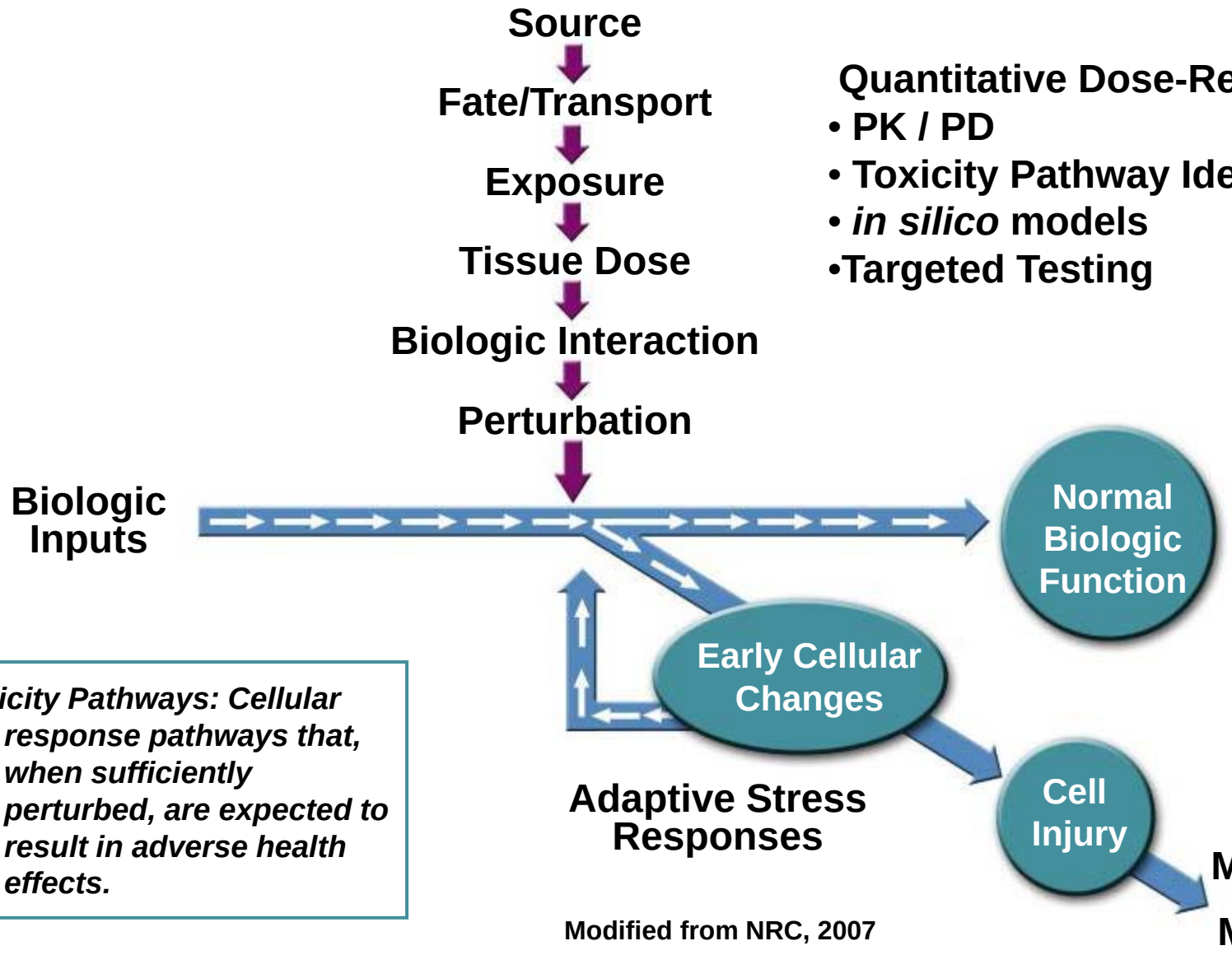
The issue

- 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



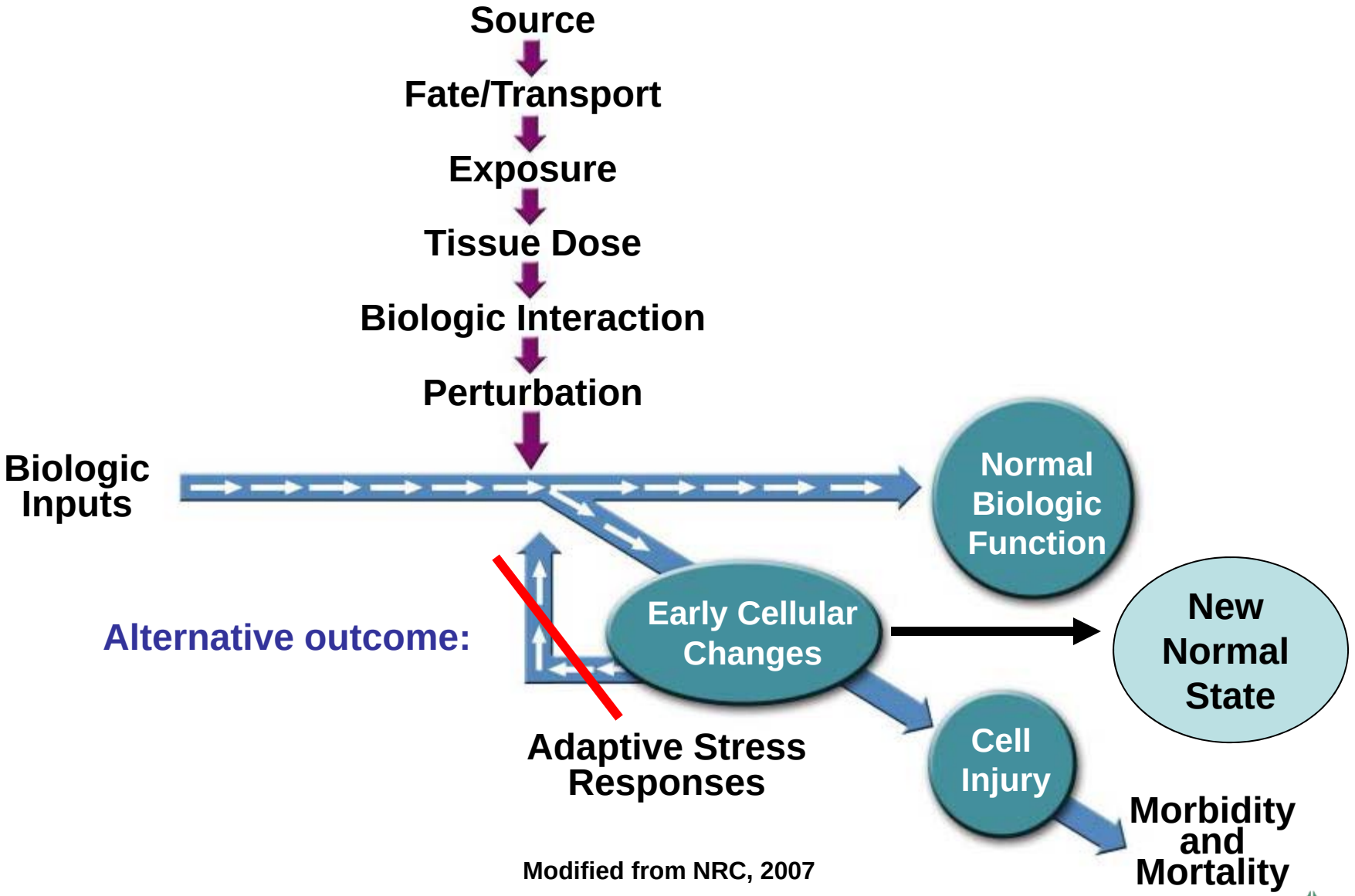
Quantitative Dose-Response

- **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.

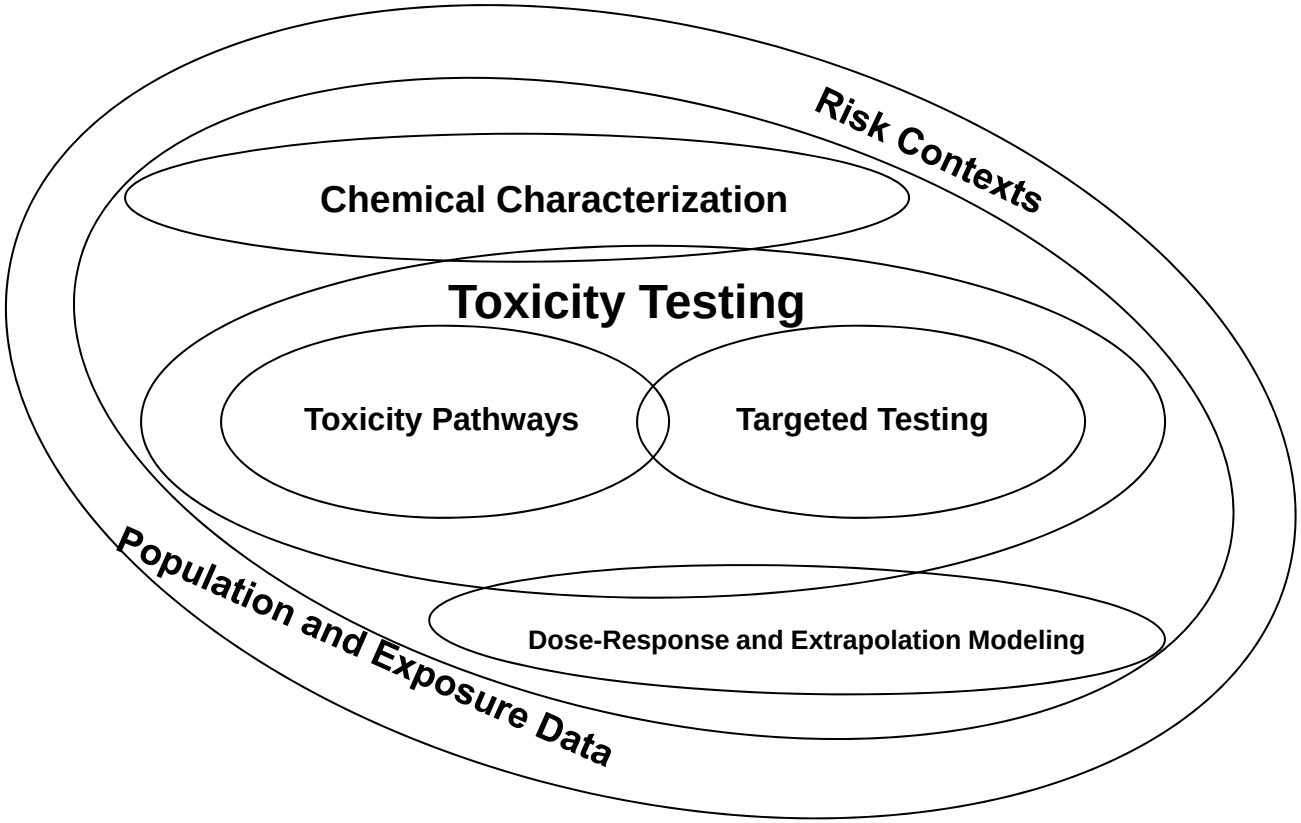
Modified from NRC, 2007

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.**

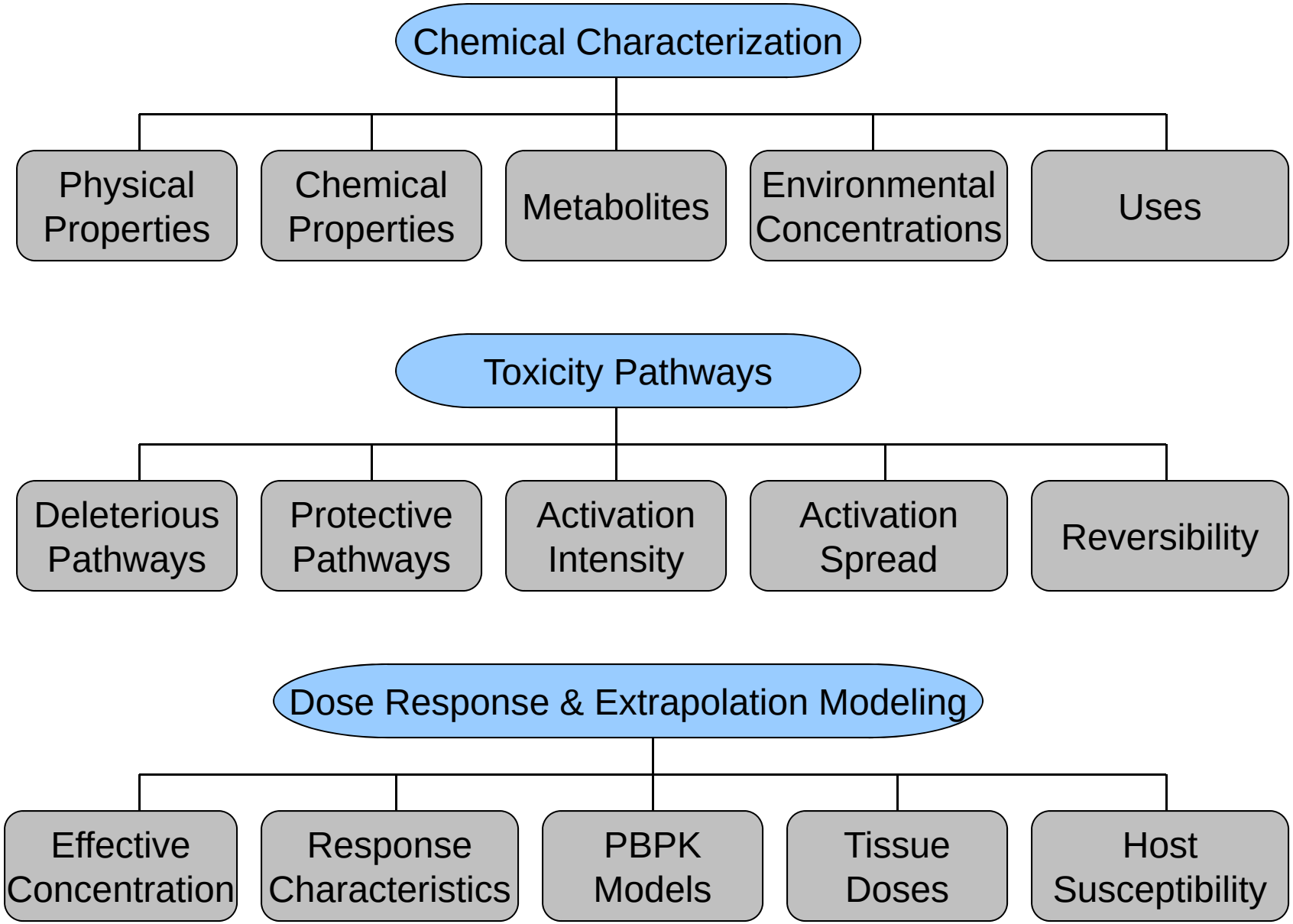
- ***Adaptive Effect:*** **In the context of toxicology, the process whereby a cell or organism responds to a xenobiotic so that the cell or organism will survive in the new environment that contains the xenobiotic without impairment of function.**

The framework – first pass

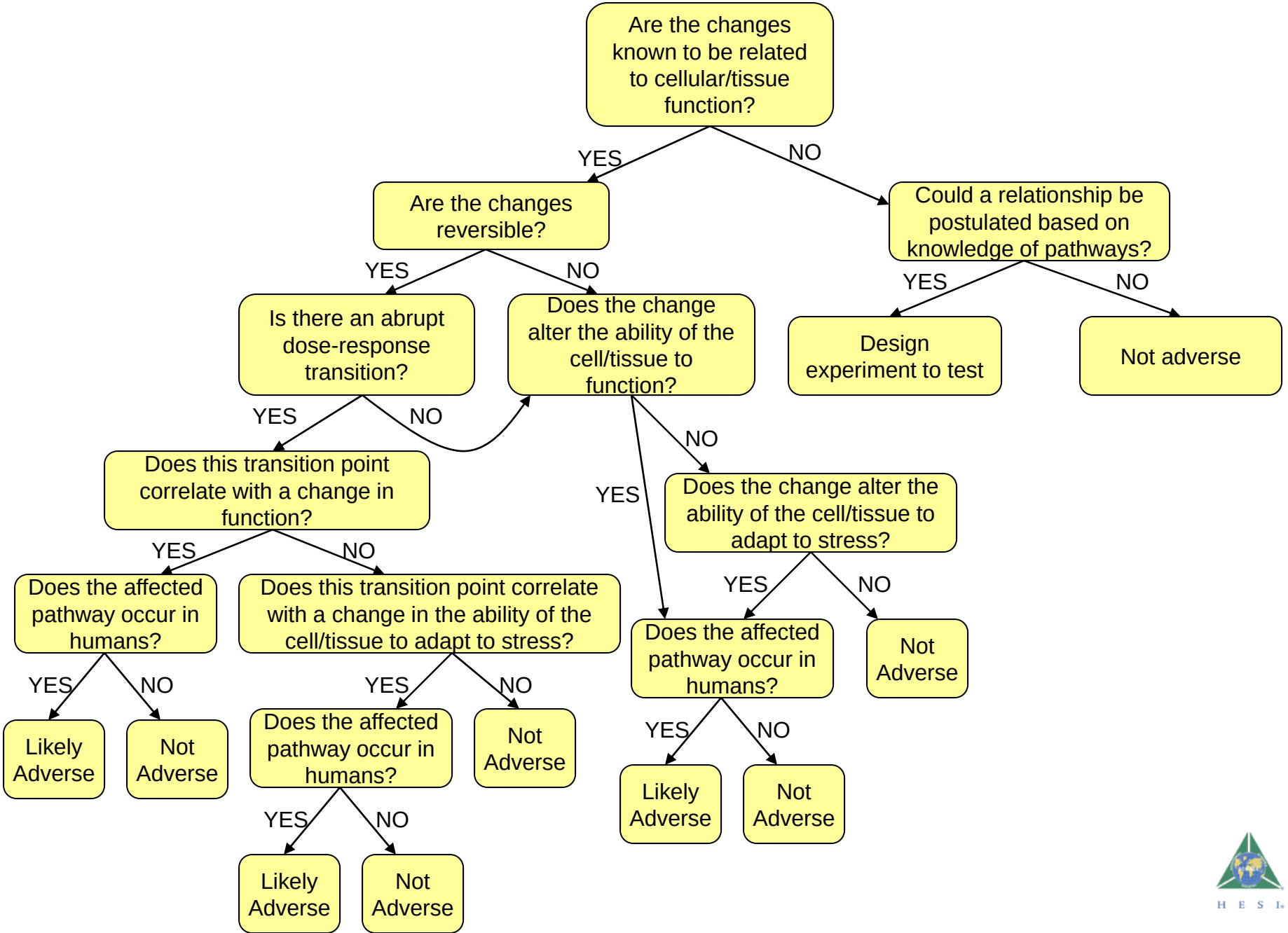
- **Characterization of the effect**
  - What is the observation? Reversible? Dose-response? Etc.
- **Relative placement of the effect to other levels of biological organization**
  - Key event in known or postulated MOA? Known to be associated with altered organ/tissue/system function? Depleted physiological 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

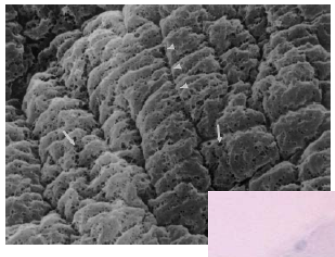
- **Acetaminophen hepatic toxicity**
  - Rich data set of histopathology, clinical chemistry, biochemical endpoints, toxicogenomics
  - Known human relevance
- **Dimethylarsenate urinary bladder carcinogenicity**
  - Rich data set of histopathology, in vitro cytotoxicity, biochemical endpoints, toxicogenomics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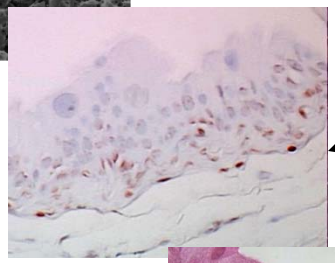

(sustained) **Enhanced Cell Proliferation**

BrdU Labeling

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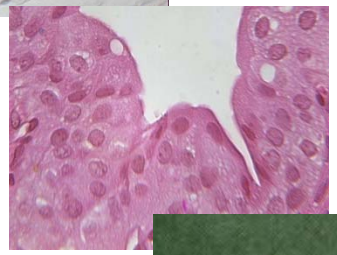

Hyperplasia

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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)

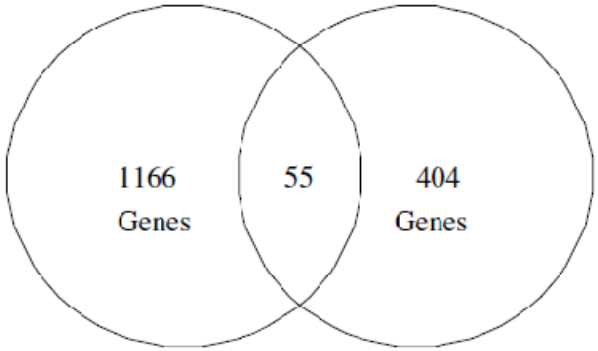


Fig. 2a. Venn analysis showing the common and unique number of significant genes across the *in vivo* and *in vitro* rat model systems.

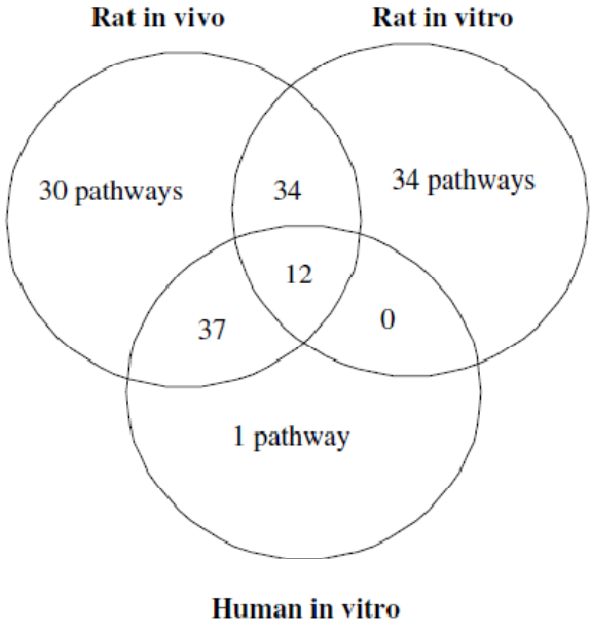
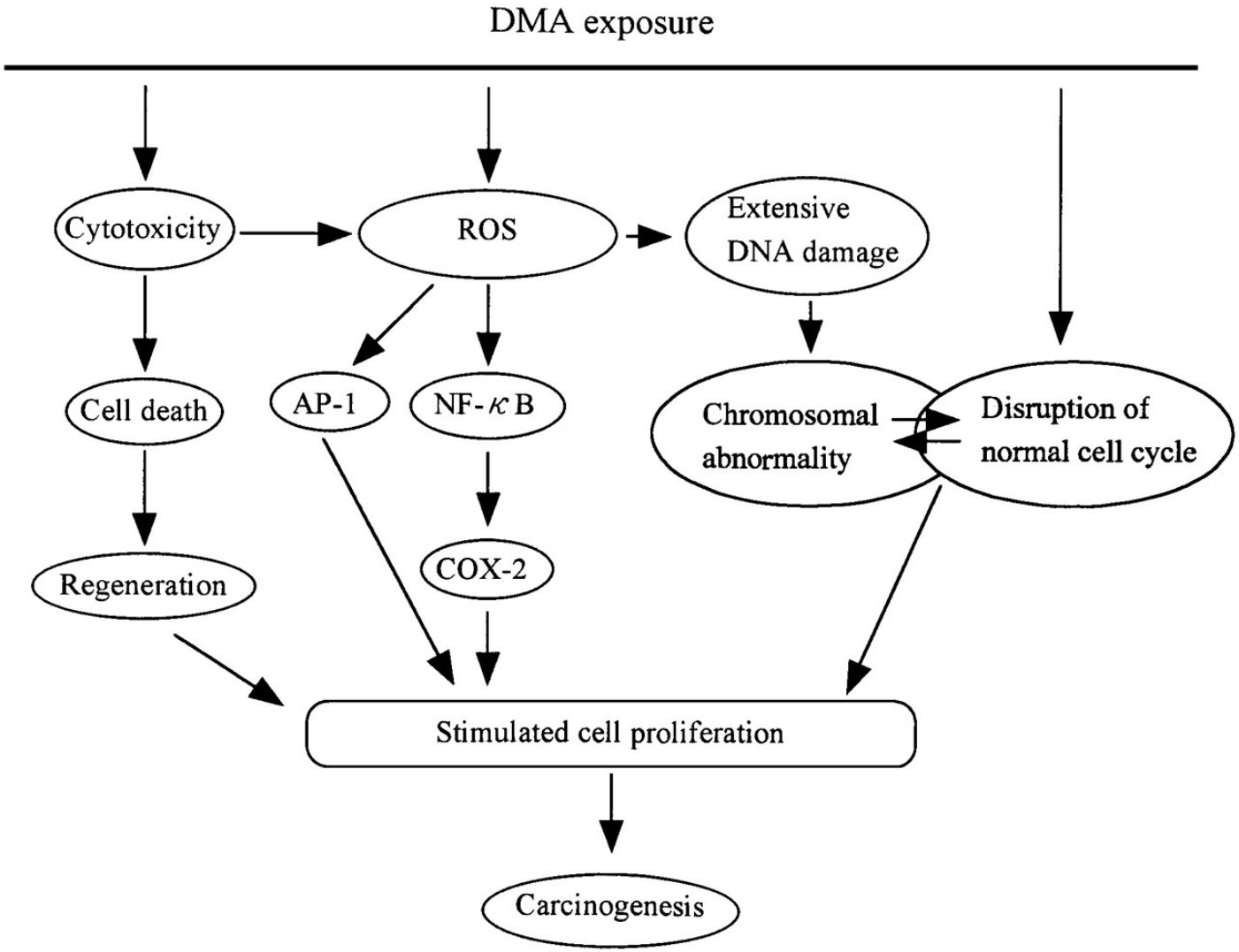


Fig. 2b. Venn analysis showing the number of common and unique significant pathways across *in vivo* and *in vitro* rat and *in vitro* human bladder model systems.

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