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Preliminary Materials for the IRIS Assessment of Hexavalent Chromium (Part II)

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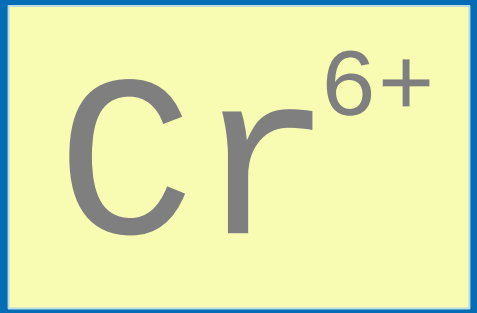

Office of Research and Development
National Center for Environmental Assessment, Integrated Risk Information System

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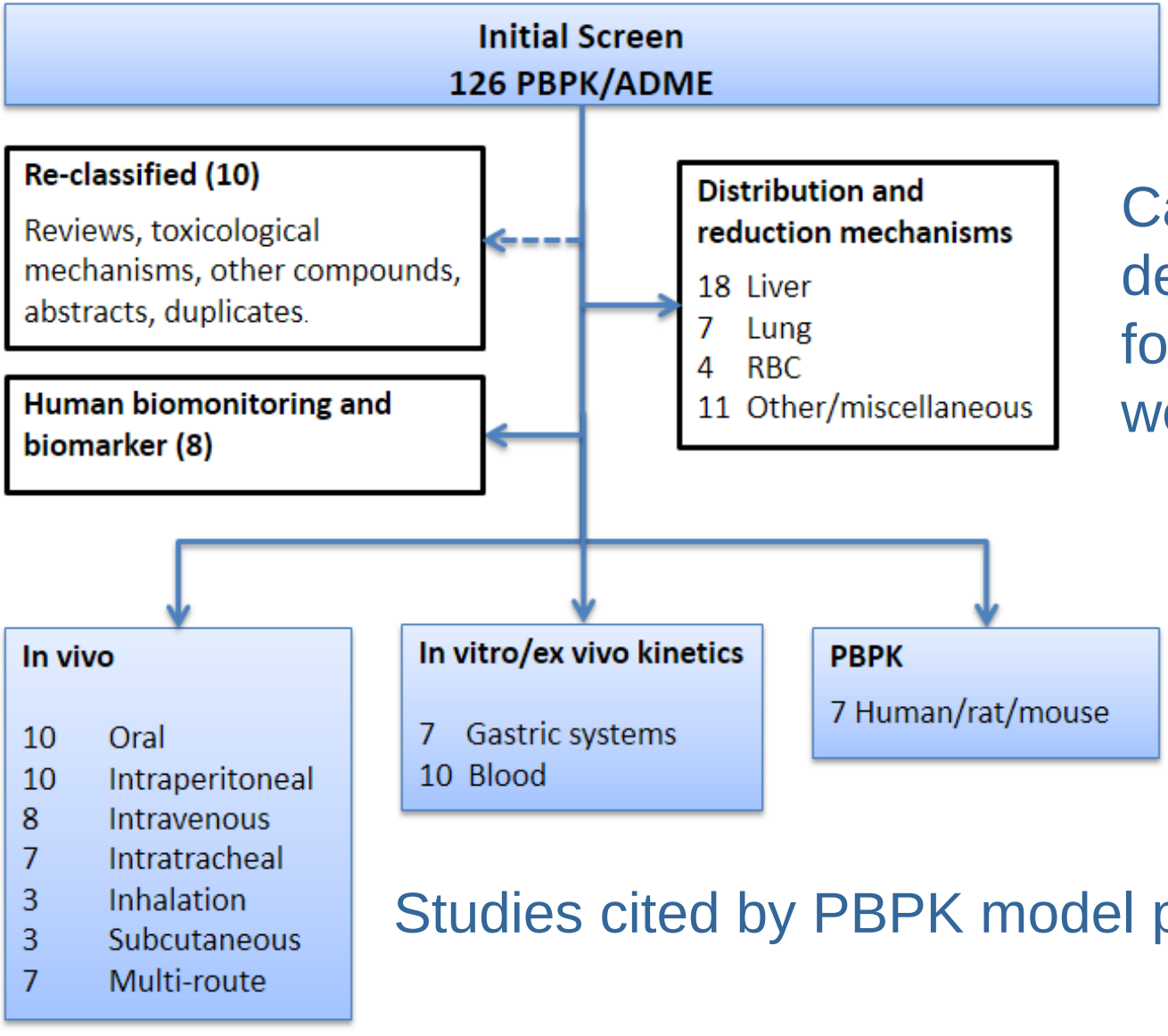
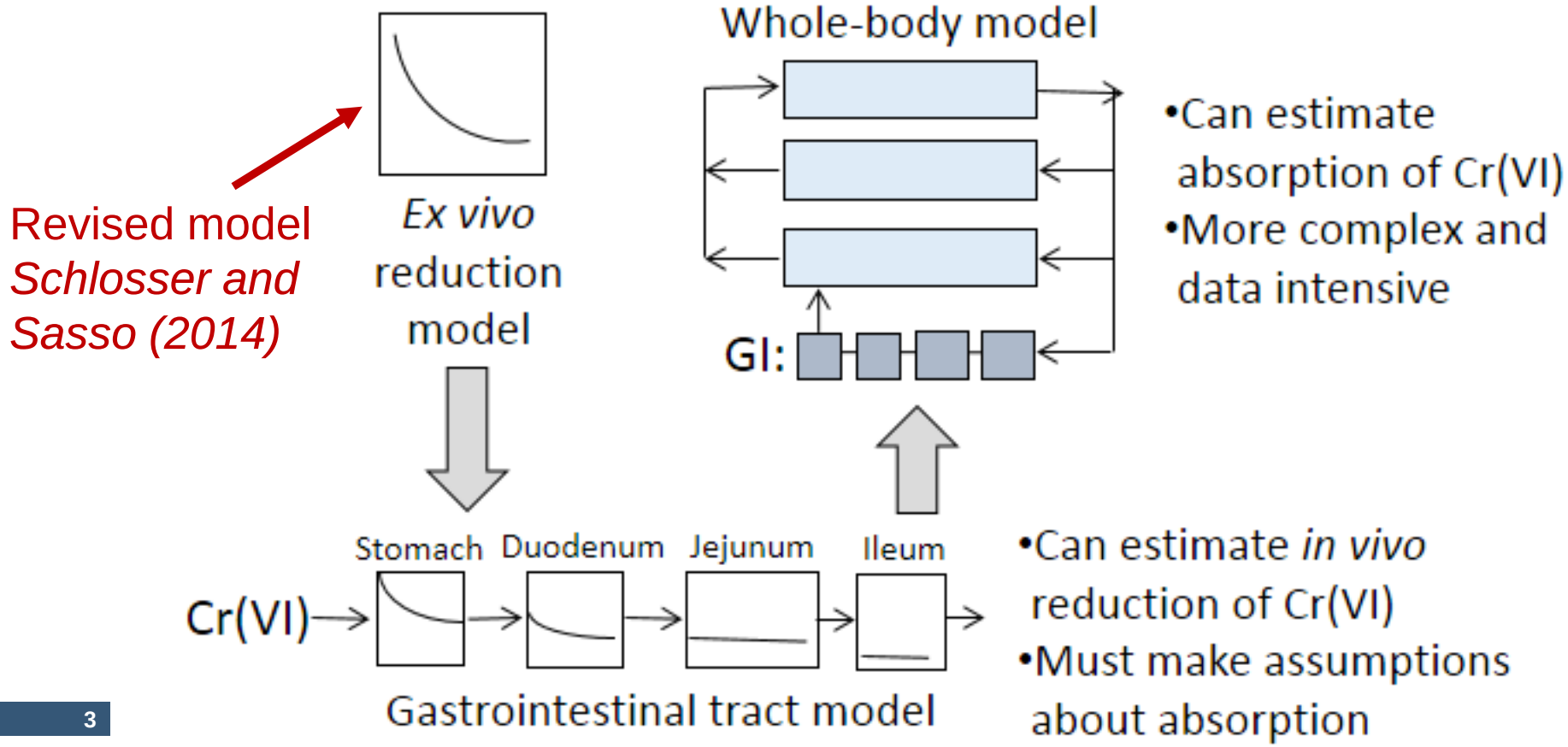
Science Question 3: Toxicokinetic considerations for dose-response



Categories determined following internal workgroup reviews

Studies cited by PBPK model papers are noted

Types of toxicokinetic models available for Cr(VI)



Science question 3: Toxicokinetic considerations for dose-response

- Aspects of the small intestine and oral cavity of mice, rats, and humans that may be significant factors in interpreting toxicity data
- Internal dose metrics that may be used for dose-response modeling
  - Mode of action and confidence in PBPK model predictions are important considerations*
- Data regarding the variability of gastric parameters in rodents and humans
- Possible contribution of gastric reducing agent depletion to nonlinear tumor response in mice at high doses of the NTP 2-year bioassay*

*New points raised by discussants

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Science Question 4: Mechanistic studies database

WHERE WE ARE NOW (PRELIMINARY PACKAGE):

Literature search and preliminary sorting of studies and endpoints

- Conducted literature search (in HERO database) that identifies mechanistic studies with primary data by keywords and brief abstract reviews
- Recorded endpoints reported in studies into a spreadsheet and assign mechanistic category to each
- Use spreadsheet to sort endpoints by mechanistic category to facilitate next phase of review

Next steps for evaluation of mechanistic studies and MOA analysis

- Following preliminary identification of health hazards, analyze hazard-specific mechanistic endpoints and collate studies reporting these endpoints
- Identify mechanistic events that result from exposure to Cr(VI)
- Evaluate hypothesized mode(s) of action and postulated key events
- Synthesize evidence and develop tables that present crucial aspects of the data
    - Inform the hazard identification
    - Identify susceptible subpopulations and lifestages
    - Inform dose-response approach

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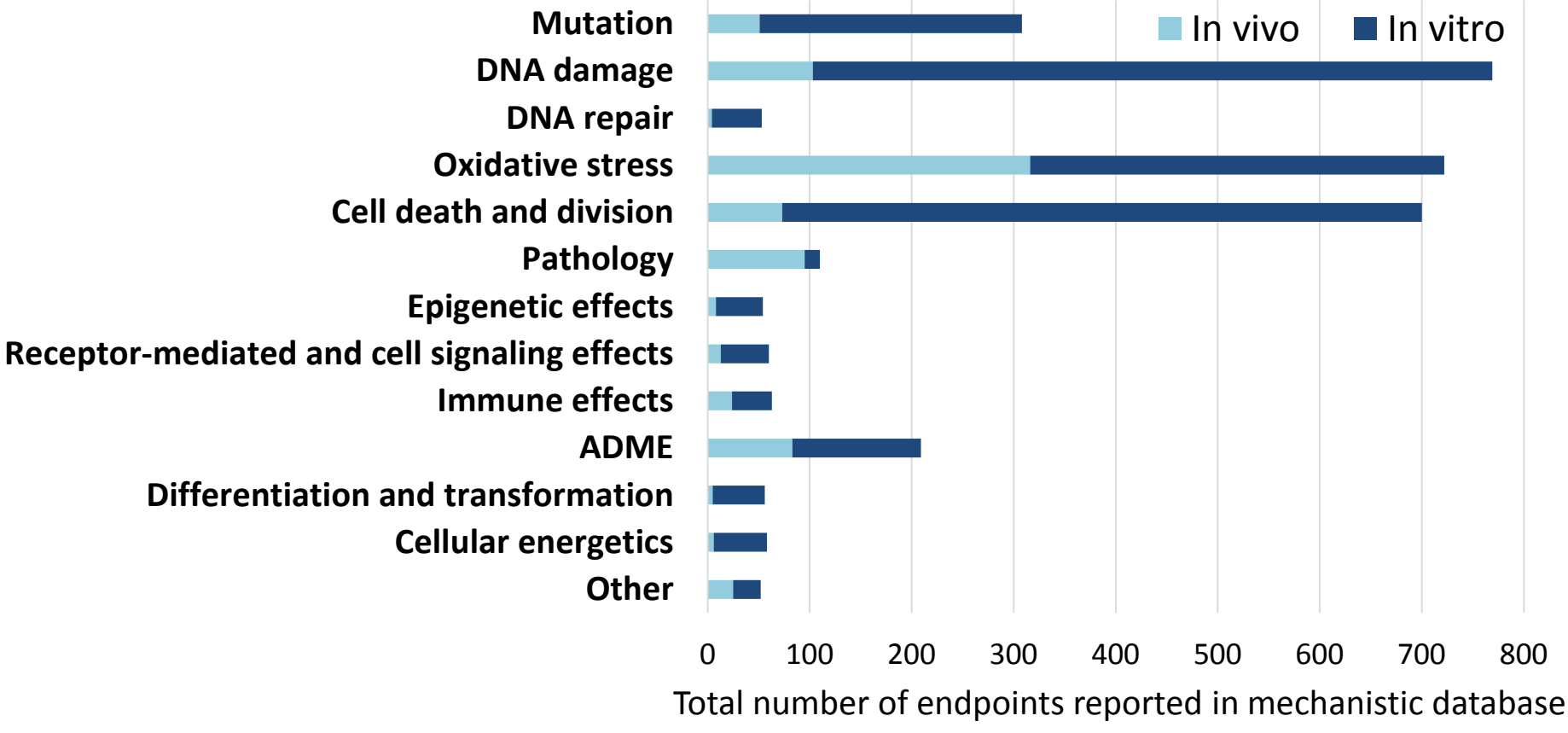
Mechanistic Categories

1. Mutation
2. DNA damage
3. Alterations of DNA repair
4. Oxidative stress
5. Changes in cell death and division
6. Pathology
7. Epigenetic effects
8. Receptor-mediated and cell signaling effects
9. Immune system effects
10. Cellular and molecular ADME
11. Cellular differentiation and transformation
12. Cellular energetics
13. Other

Inventory of mechanistic database for Hexavalent Chromium



Science question 4: Mechanistic studies database

Information from mechanistic studies of hexavalent chromium was extracted into a spreadsheet as a preliminary step in the process of database organization and analysis.

EPA seeks discussion on:

- Aspects of the database, including the preliminary designation of mechanistic categories, that could be improved
- Any studies that might be missing from this database

Science question 4 (continued)

The following related comment was suggested by Mark Harris (ToxStrategies, Inc.) on behalf of the ACC, and Ted Simon (Ted Simon LLC) on behalf of Ted Simon LLC:

Many scientists and regulators believe that mode of action should be considered very early in the evaluation process and throughout the analysis to enable effective use of data in human health risk assessment (Meek et al., 2014, Simon et al., 2014). Such consideration can focus efforts and resources on key endpoints and relevant data. For example, are the available data more consistent with a direct mutagenic or indirect nonmutagenic mode of action in the etiology of hexavalent chromium-induced tumors in the small intestine of mice?

Two hypotheses exist in the scientific literature to explain the observed dose-dependent increase in small intestinal tumors in mice exposed to hexavalent chromium in drinking water (McCarroll et al., 2010; Thompson et al., 2011). Such tumors were not observed in similarly exposed rats. Discussion is sought on the modes of action proposed in these papers and on the utility of considering them at different stages in the assessment process.

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Science Question 5: Chromium-DNA adducts

Major Cr-DNA adducts

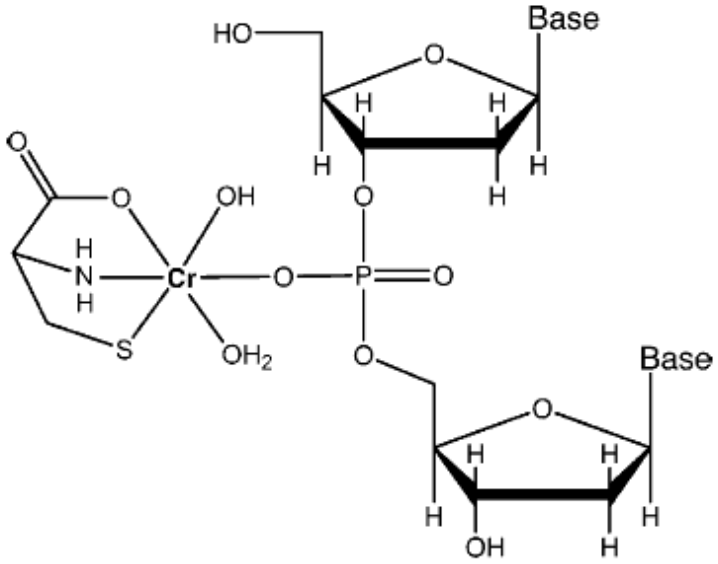

Figure 4. Proposed structure of cysteine−Cr(III)−DNA cross-link. This adduct is the most abundant Cr−DNA modification in Cr(VI)-treated cells (*52*). Tridentate coordination of cysteine to Cr(III) has been determined by analysis of crystal structures of bis(L-cysteinato)chromium(III) complexes (*85*, *86*).

Zhitkovich, *Chem Res Toxicol* 2005

Major Cr-DNA adducts (continued)

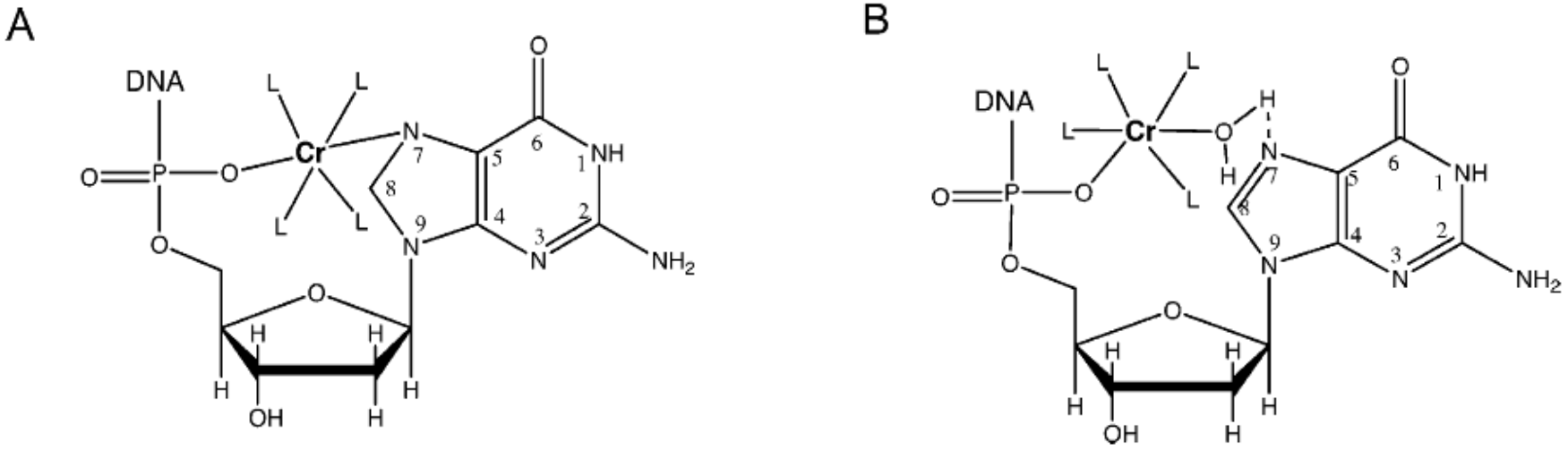

Figure 5. Formation of microchelates (intramolecular cross-links) by DNA-bound chromium(III). (A) Direct coordination of Cr(III) to 5′-phosphate and N-7 of dG. This mode of DNA binding is likely to be limited to small binary Cr(III)−DNA adducts (*76*). (B) Direct coordination of Cr(III) to 5′-phosphate and hydrogen bonding to N-7 of dG. This binding mode can occur for both binary and ternary Cr(III)−DNA adducts.

Zhitkovich, *Chem Res Toxicol* 2005

Science question 5: Chromium-DNA adducts

The ability of chromium (in the trivalent oxidation state, intracellularly) to covalently bind DNA molecules has been shown by several investigators experimentally in vitro. However, these adducts have not been observed in vivo.

EPA is seeking public discussion on the feasibility of chromium-DNA adducts forming in vivo and how predictive these lesions are of mutagenic potential.

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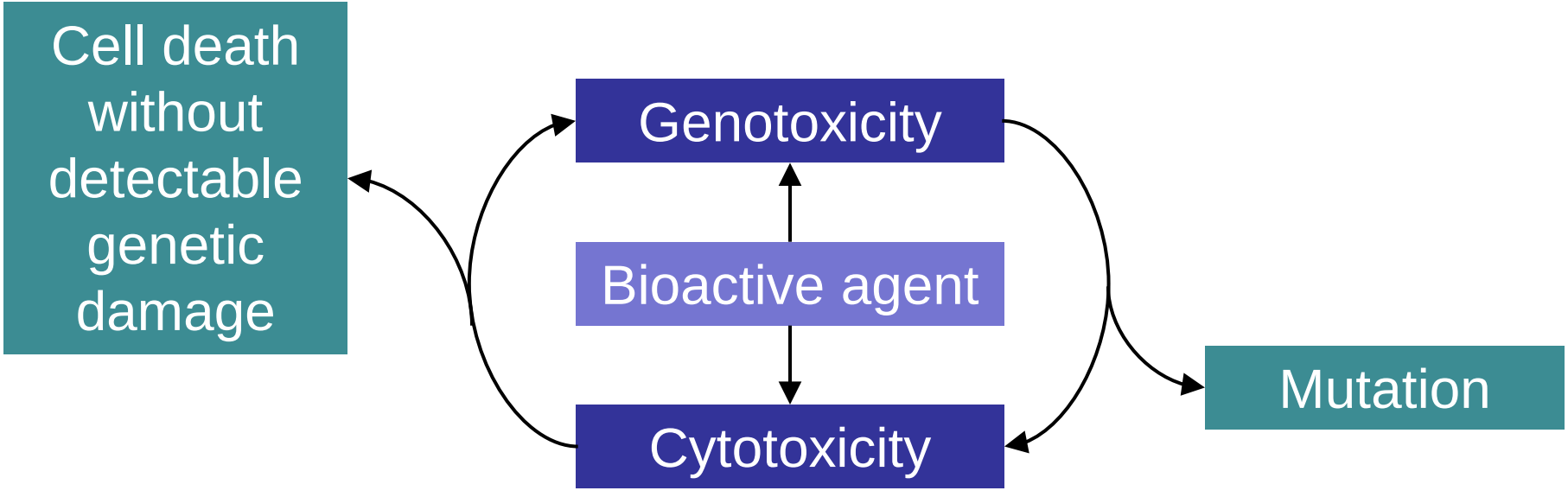
Science Question 6: In vitro mutagenicity/genotoxicity studies

Interrelationship between Cytotoxicity and Genotoxicity



- Underscores the importance of consideration of cytotoxicity measures when interpreting study findings
- Cytotoxicity guidance exists for in vitro genotoxicity assays (ICH, OECD)

Figure courtesy of David Eastmond

Mechanistic Categories

1. Mutation
2. DNA damage
3. Alterations of DNA repair
4. Oxidative stress
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Science question 6: In vitro mutagenicity/genotoxicity studies

In 2010, EPA released an external review draft IRIS assessment for hexavalent chromium. In the final peer review report (2011), it was emphasized by several reviewers that for in vitro studies of mutagenicity and genotoxicity, positive results are only observed following very high concentration exposures that are toxic to the cells exposed.

In vitro studies often rely on using high exposures to induce effects to allow experimental investigation of mechanistic events. EPA is seeking discussion of the utility of these studies to inform mechanistic evaluation for hexavalent chromium.

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Science Question 7: New issue suggested by Deborah Proctor (ToxStrategies, Inc.) on behalf of the Electric Power Research Institute (EPRI)

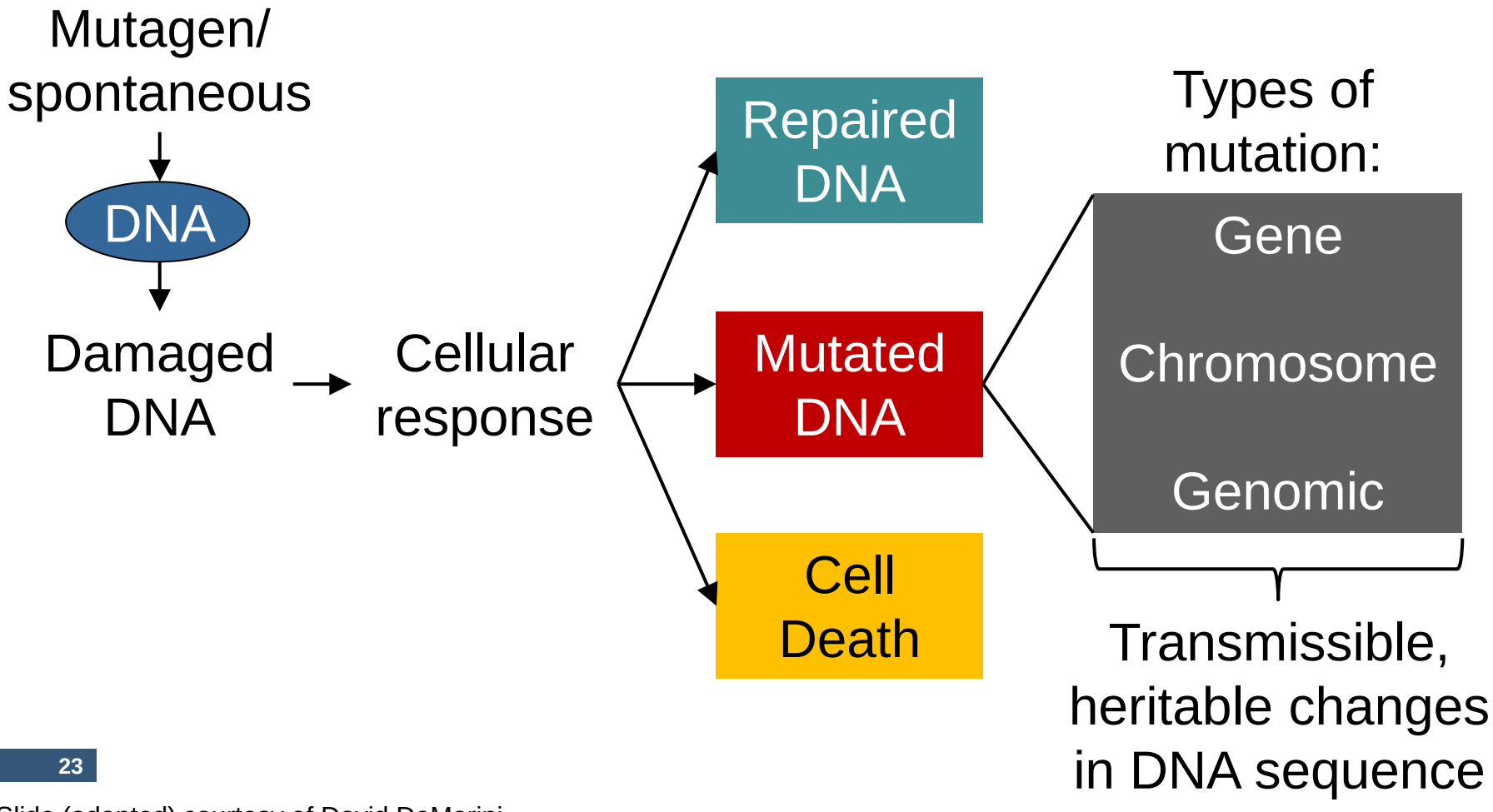
Science question 7: New issue suggested by Deborah Proctor (ToxStrategies, Inc.) on behalf of the Electric Power Research Institute (EPRI):

Several in vivo genotoxicity/mutagenicity assays have been conducted for hexavalent chromium, including in target tissues of carcinogenicity (e.g. intestine). Some of these in vivo studies have employed high, carcinogenic concentrations by relevant routes of exposure and have indicated negative results with respect to genotoxicity/mutagenicity.

Discussion is sought of the utility of these studies to inform mechanistic evaluation for hexavalent chromium.

Science Question 8: Definitions of mutagenicity and genotoxicity

Mutagenesis paradigm



Slide (adapted) courtesy of David DeMarini

EU Technical Guidance on Risk Assessment (1996)

"The chemical and structural complexity of the chromosomal DNA and associated proteins of mammalian cells, and the multiplicity of ways in which changes to the genetic material can be affected, make it difficult to give precise, discrete definitions.

- **Mutagenicity** refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. These changes, 'mutations,' may involve a single gene or gene segment, a block of genes, or whole chromosomes. Effects on whole chromosomes may be structural and/or numerical.
- **Genotoxicity** is a broader term and refers to potentially harmful effects on genetic material, which may be mediated directly or indirectly, and which are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include tests which provide an indication of induced damage to DNA (but not direct evidence of mutation) via effects such as unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE) or mitotic recombination, as well as tests for mutagenicity."

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Definition used by the World Health Organization (WHO) International Programme on Chemical Safety (IPCS) Harmonized Scheme for Mutagenicity Testing

- "…'**Mutation**'…refers to permanent changes in the structure and/or amount of the genetic material of an organism that can lead to heritable changes in its function, and it includes gene mutations as well as structural and numerical chromosome alterations.
- '**Genotoxicity**' refers to the capability of substances to damage DNA and/or cellular components regulating the fidelity of the genome—such as the spindle apparatus, topoisomerases, DNA repair systems and DNA polymerases—and includes all adverse effects on genetic information."

(Eastmond et al., *Mutagenesis,* 2009)

EPA Guidelines for Carcinogen Risk Assessment (2005)

"Key data for a mutagenic mode of action may be evidence that the carcinogen or a metabolite is DNA-reactive and/or has the ability to bind to DNA. Also, mutagenic carcinogens usually produce positive effects in multiple test systems for different genetic endpoints, particularly gene mutations and structural chromosome aberrations, and in tests performed *in vivo* which generally are supported by positive tests *in vitro*. Additionally, carcinogens may be identified as operating via a mutagenic mode of action if they have similar properties and SAR to mutagenic carcinogens."