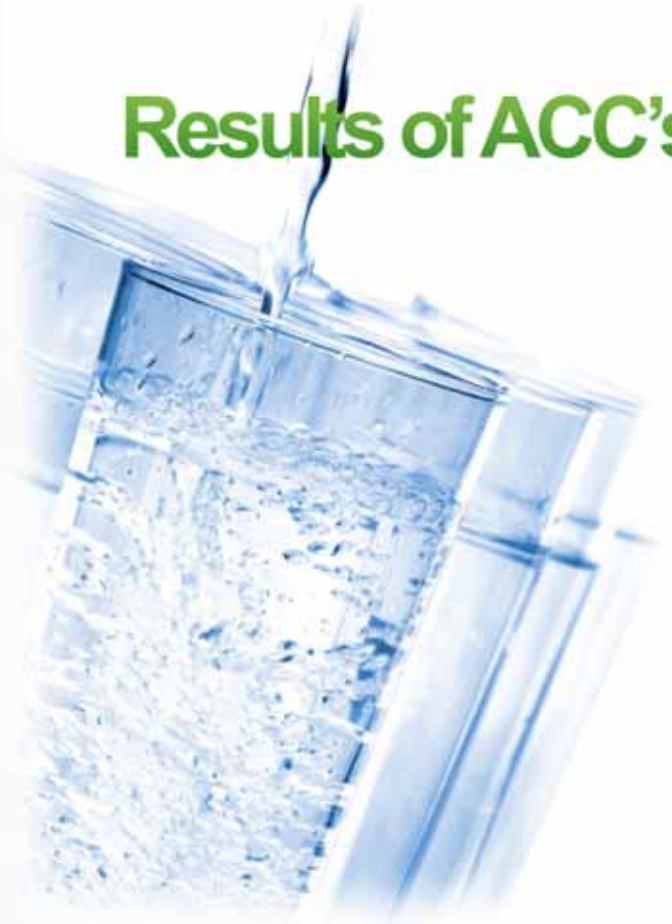
Do Low Concentrations of Hexavalent Chromium in Drinking Water Pose a Cancer Hazard?

Results of ACC's Cr(VI) Mode of Action Study



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Chris Kirman and Sean Hays

ToxStrategies, Inc. and Summit Toxicology

November 2012

Research Project funded by the Cr 6 Panel of the American Chemistry Council

ToxStrategies

Presentation Overview

- Background regarding Cr(VI) toxicology and the Mode of Action (MOA) Research Project
- Research findings that inform the risk assessment for relevant drinking water exposure (i.e., by humans at low levels)
- How kinetic models[1] and “mode of action” data are used in cancer risk assessment to set safe levels
- Calculation of Drinking Water Equivalent Level (DWEL) protective of intestinal cancer

[1]Physiologically-based pharmacokinetic (PBPK) models

Cr(VI) MOA Study Research Team

Universities

George Washington University Medical Center
Michigan State University
University of Cincinnati Medical Center
Duke University Medical School

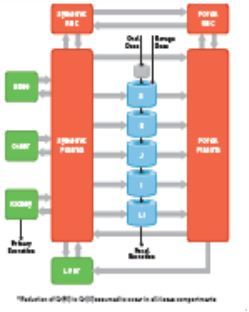
Analytical Laboratories

Applied Speciation
Brooks Rand Laboratory
Environmental Standards

Research Laboratories

Experimental Pathology Laboratories
Southern Research Institute
National Center for Toxicological Research
ThermoFisher

Risk Assessors and Modelers



Summit Toxicology
ToxStrategies

National Toxicology Program (NTP) Study Results for Cr(VI) and Cr(III)

NTP Cr(VI) drinking water study

- Mice and rats consuming 5,000 – 180,000 µg/L (ppb) Cr(VI) as sodium dichromate dihydrate (SDD)
- Rare tumors appeared late in the study
  - Mice: adenomas and carcinomas of small intestines
  - Rats: squamous cell carcinoma in oral cavity

B6C3F1 mouse

F344/N rat

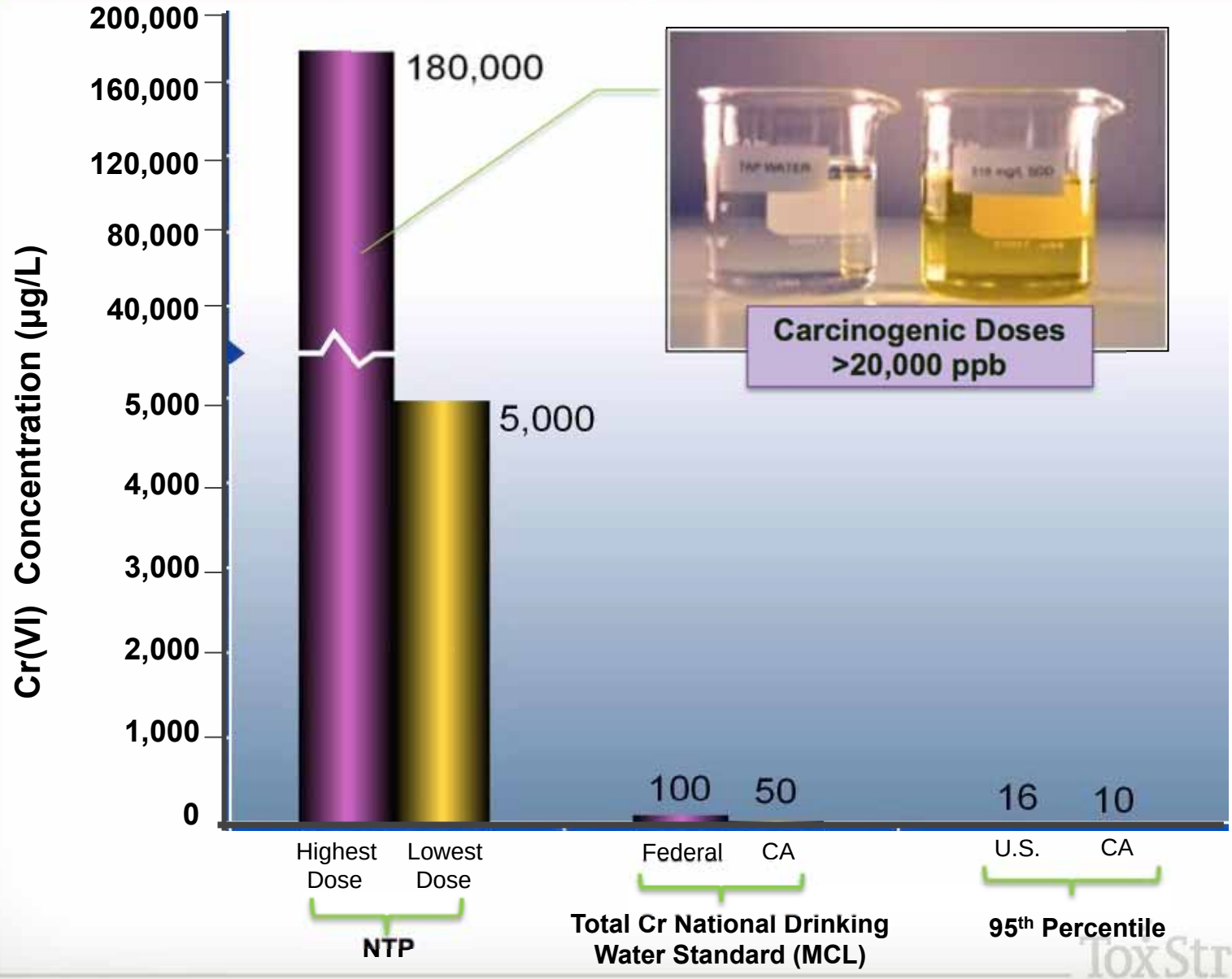
NTP Cr(III) drinking water study

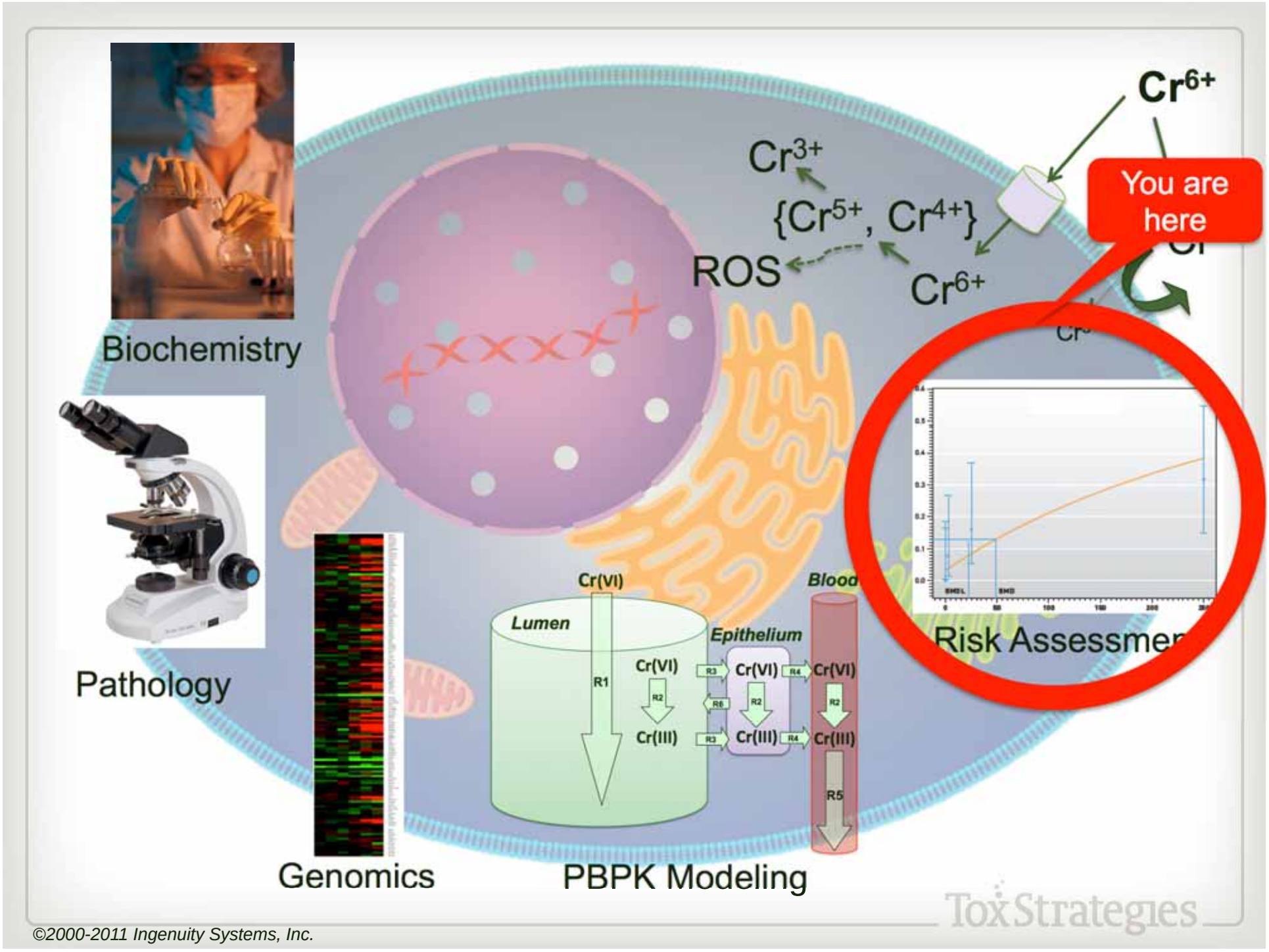
- No significant effects observed in either species

Cr(VI) MOA Research Project Background

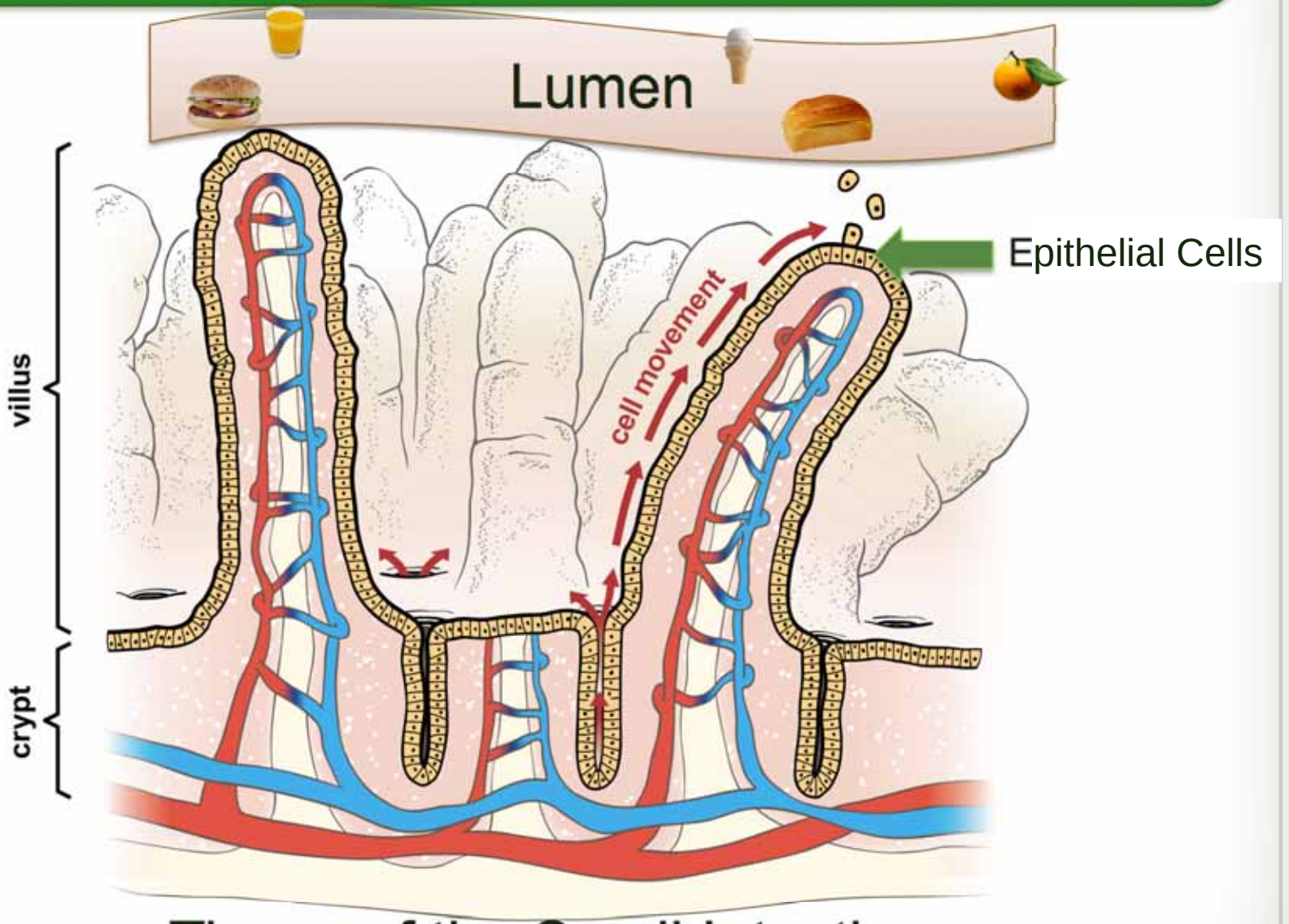
- The Cr(VI) MOA research project was developed using EPA Guidance
- Provides information as to why tumors occurred in rodents
- Provides information on the differences between rodents and humans with regard to internal dose
- Develops the models and data needed to do a State-of-the-Art Risk Assessment

Comparison of NTP Doses to Human Exposures



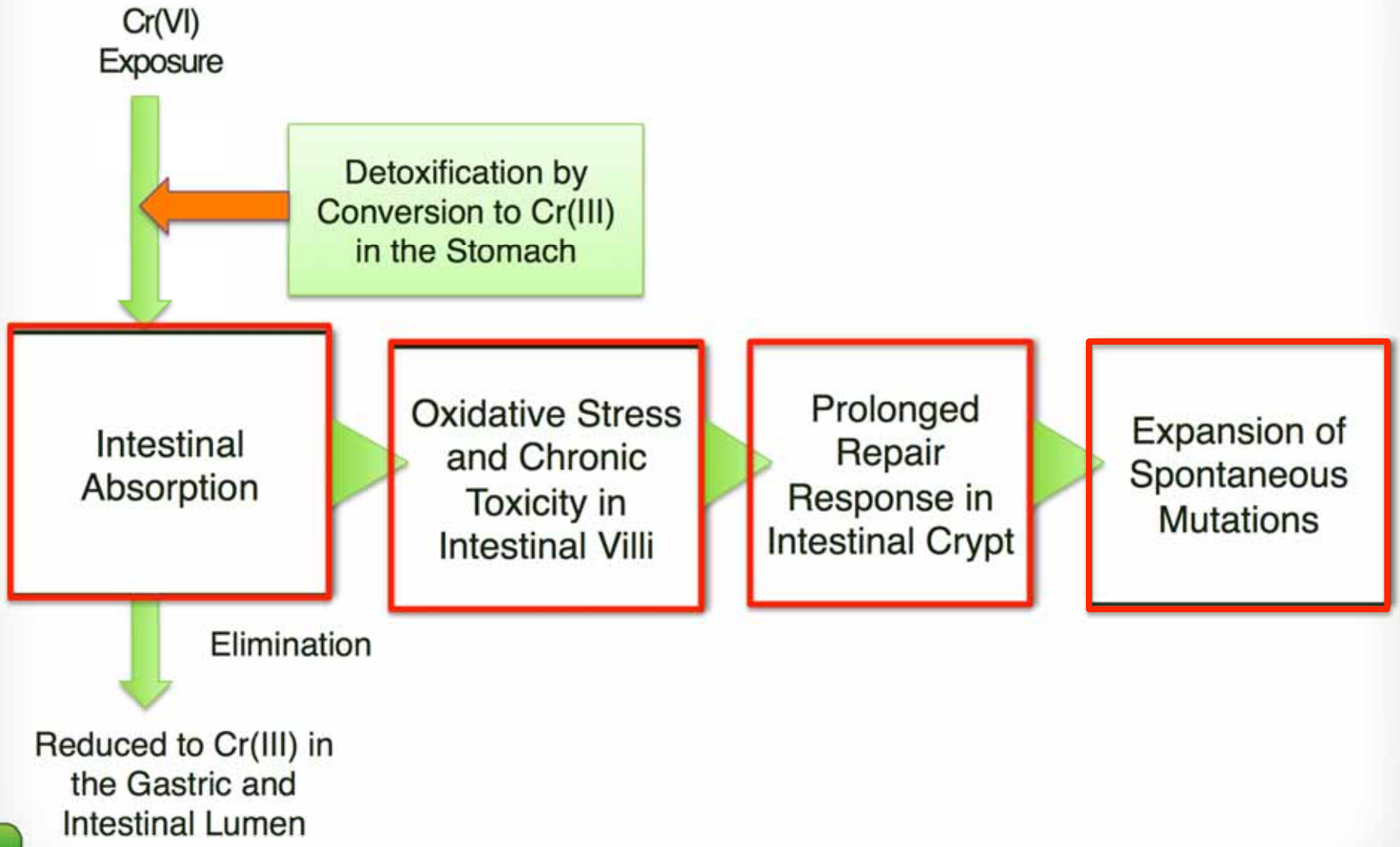


Biology is Important



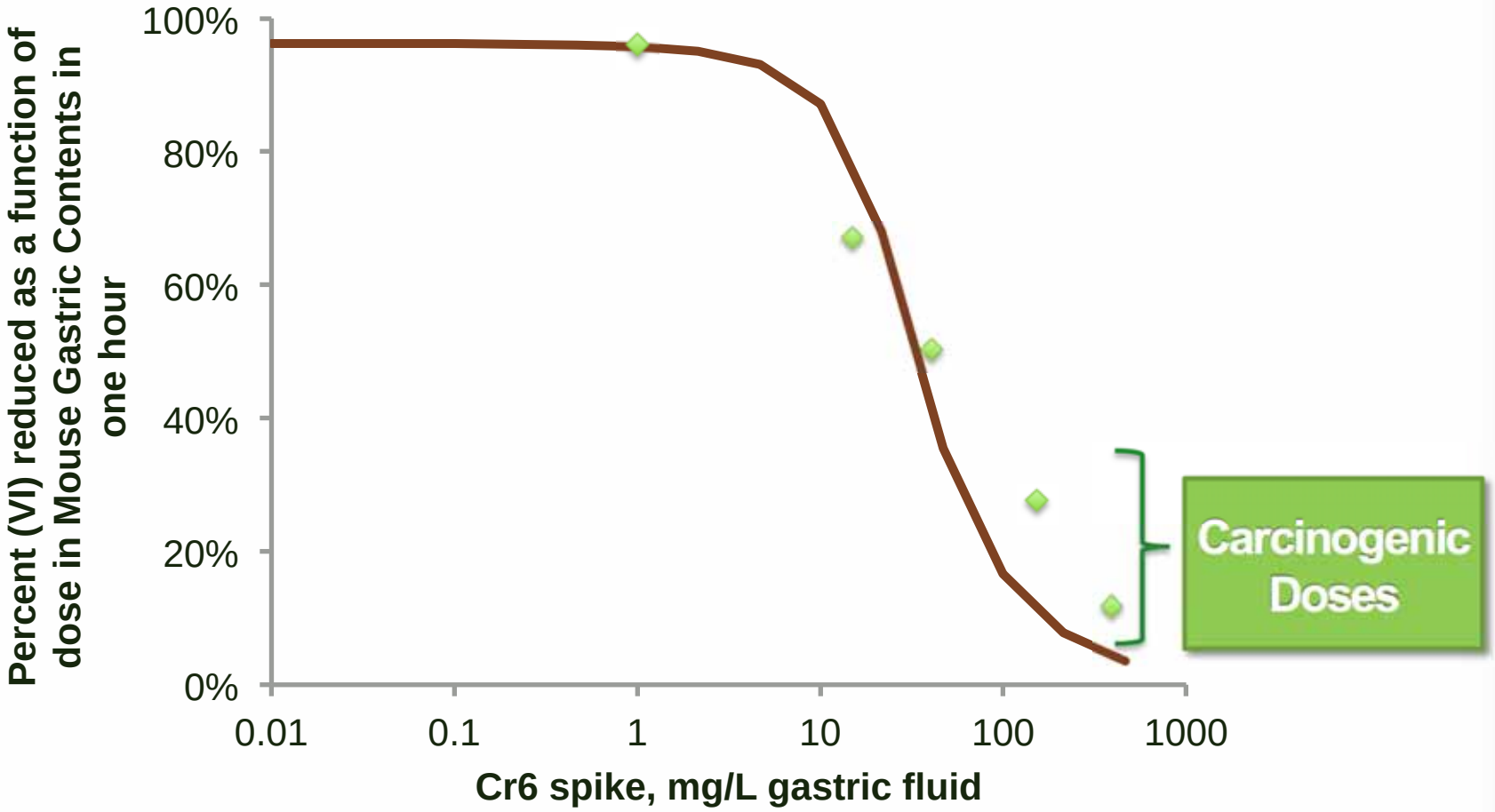
Tissue of the Small Intestine

Mode of Action for Intestinal Cancer



Stomach Reduction Kinetics

Stomach Reduction Capacity is Exceeded At Carcinogenic Doses in Rodents

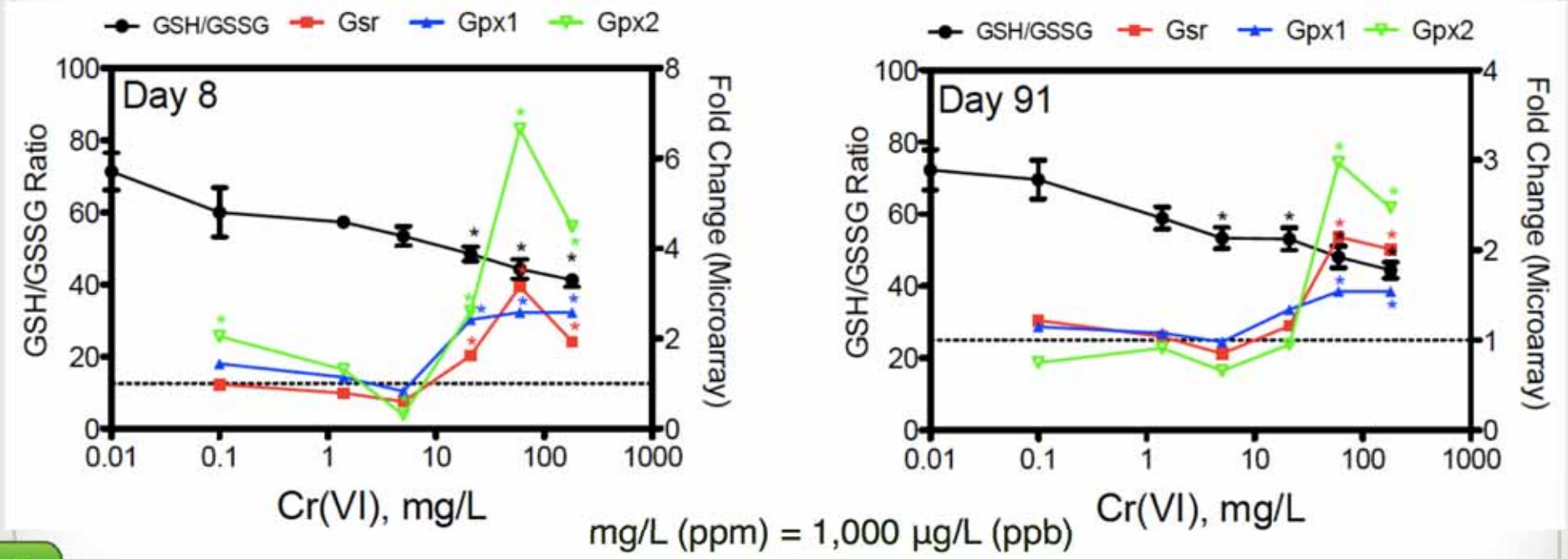


Data by Applied Speciation

Oxidative Stress and Chronic Toxicity in Intestinal Villi

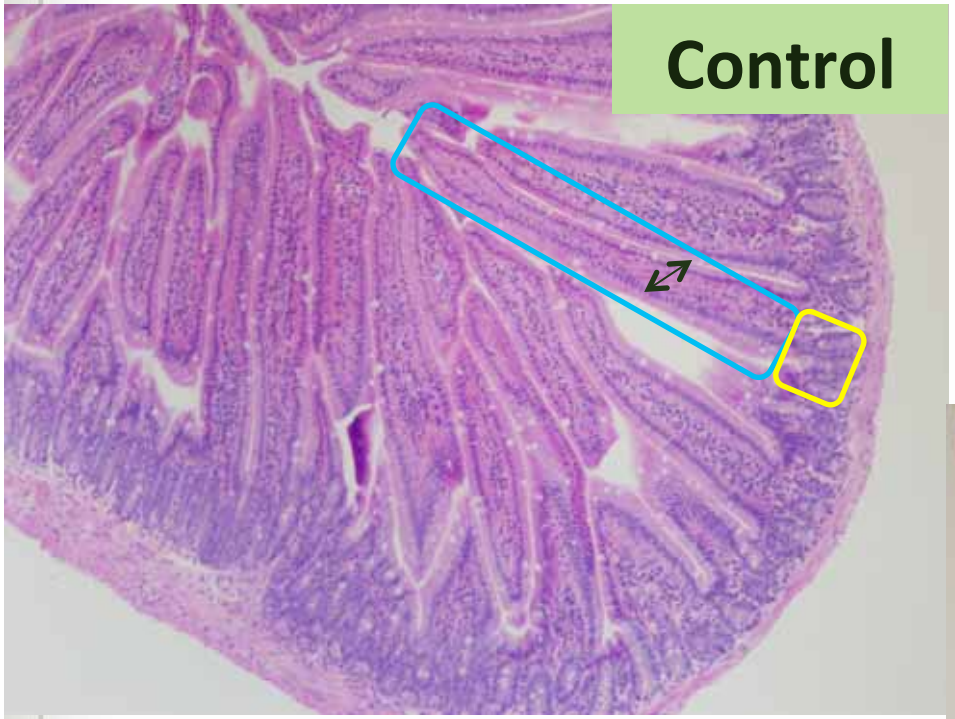
Biochemical and Genomic Responses to Oxidative Stress

- Significant decreases in reduced to oxidized glutathione in mouse duodenum and jejunum
- Activation of genomic response to oxidative stress



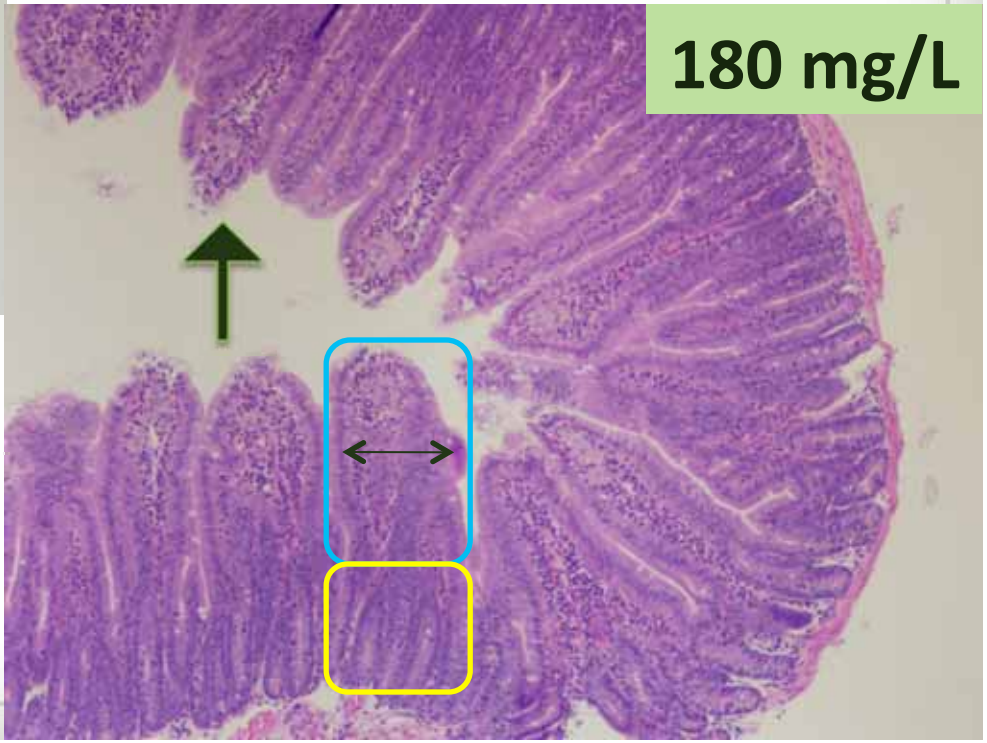
Toxicogenomics by Michigan State; GSH/GSSG data by University of Cincinnati

Toxicity in Villus & Regeneration of New Cells in Crypt

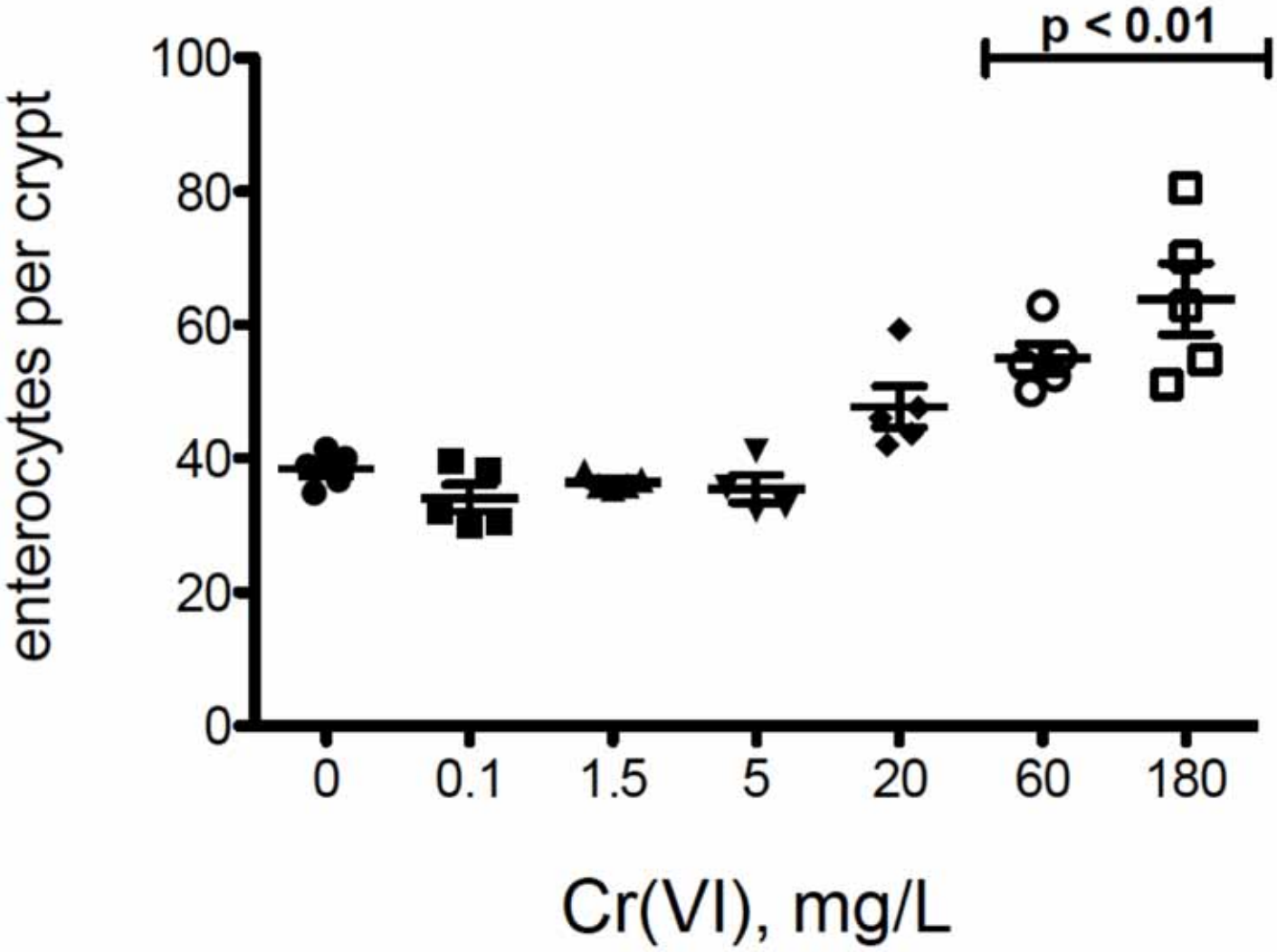


At High Doses:

- Expanded Crypt Area, Blunted Villi
- Damage at villi tips



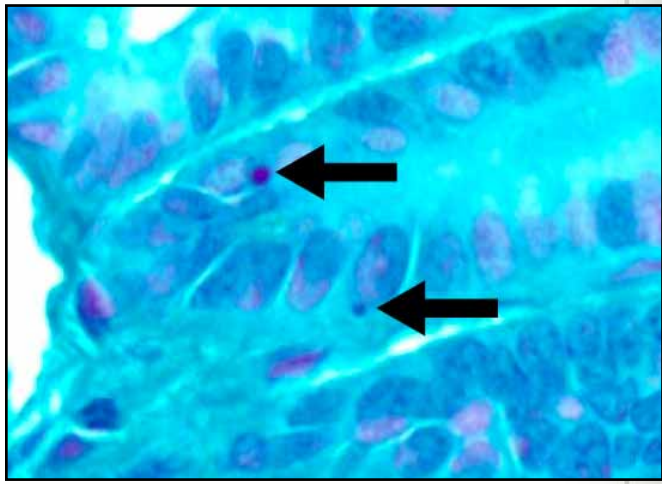
Total number of Epithelial Cells in the Duodenal Crypt (Mouse Day 90)



Does Cr(VI) Cause DNA Mutations in the Crypt or Do Tumors Occur by Spontaneous Replication Error?

Toxicity and DNA Damage to Cells in the Duodenal Crypt

- Mitotic Index: Percentage of Cells Undergoing Division
- Apoptotic Index: Percent of cells undergoing apoptosis (programmed cell death)
- Micronuclei: Total number of cells with an extra smaller nucleus indicating broken chromosomes



Measured in 10 fully intact crypts per animal,
5 animals per dose

No Toxicity to Cells in the Duodenal Crypt
(Mice Day 91)

Cr(VI) Drinking Water (mg/L)	Mitotic Index (%)		Apoptotic Index (%)		Total Number of Micronuclei
0	1.43	±1.17	0.47	±0.22	0
0.1	2.28	±1.07	1.0	±0.47	0
1	2.36	±0.684	0.5	±0.4	
5	3.08	±0.46	0.7		
20	2.46	±0.76	0.5	±0.3	
60	2.72	±0.97	0.84	±0.96	0
180	2.11	±1.09	0.67	±0.33	0

No Effect on Normal Cell Generation or Cellular Death

Purple for Carcinogenic Doses

Mitotic and apoptotic indices are percent of mitotic and apoptotic cells per total cells evaluated

Data represent total number of cells evaluated in 10 fully intact crypts per animal, 5 animals per dose group

No DNA Damage in Duodenal Crypt
(Mice Day 91)

Cr(VI) Drinking Water (mg/L)	Mitotic Index (%)		Apoptotic Index (%)		Total Number of Micronuclei
0	1.43	±1.17	0.47	±0.22	0
0.1	2				0
1	2				0
5	3				0
20	2				0
60	2				0
180	2.11	±1.09	0.67	±0.33	0

No Evidence of DNA damage in Target Crypt Cells with Proliferation Response

Purple for Carcinogenic Doses

Mitotic and apoptotic indices are percent of mitotic and apoptotic cells per total cells evaluated

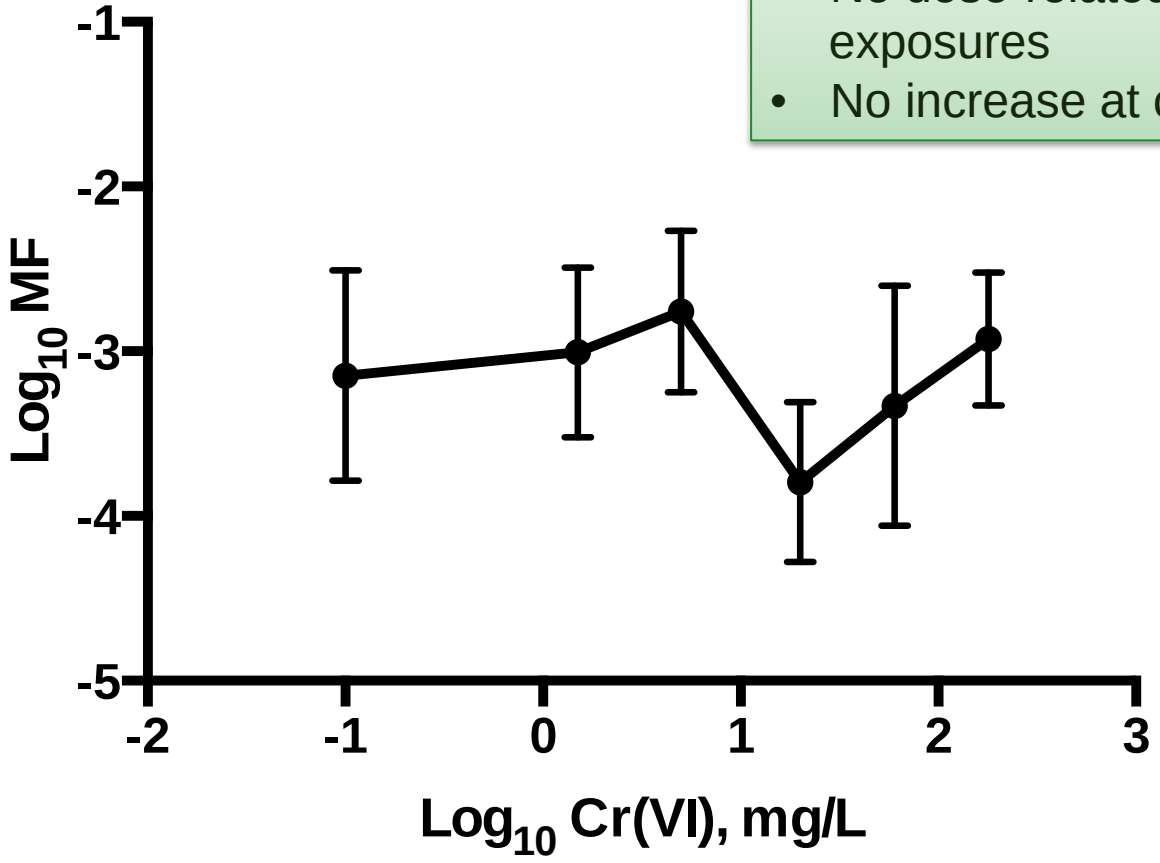
Data represent total number of cells evaluated in 10 fully intact crypts per animal, 5 animals per dose group

Mutation Analysis

- When DNA is damaged, cells die, DNA is correctly repaired, or more rarely it is repaired with an incorrect code
- If the incorrect code is in an important gene sequence (an oncogene), the cells can start to divide uncontrollably, and this is called mutagenesis
- Thus, there is an important distinction between genotoxicity (damage to DNA), and mutagenesis which is a heritable change in the DNA sequence
- We looked for a specific mutation in an oncogene (K-Ras) in mouse intestinal tissue with a very sensitive method at doses that cause hyperplasia
- K-Ras codon 12 is commonly mutated in intestinal cancers
- K-Ras codon 12 GAT mutation is also a “reporter gene” for mutations in other parts of the DNA sequence
- Mutation data, such as this, are EPA’s highest tier of data for assessing whether a chemical acts by a mutagenic MOA

K-Ras Codon 12 GAT Mutations (Mouse Duodenum, Day 91)

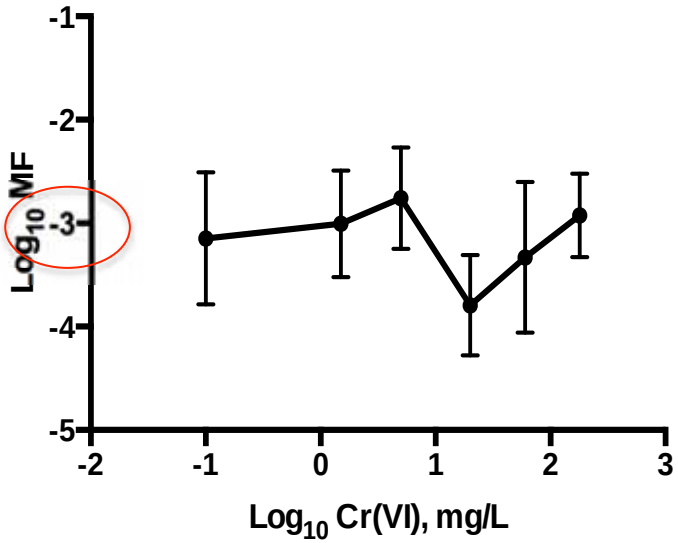
- K-Ras commonly mutated in intestinal cancers
- No dose-related trend with Cr(VI) exposures
- No increase at carcinogenic doses



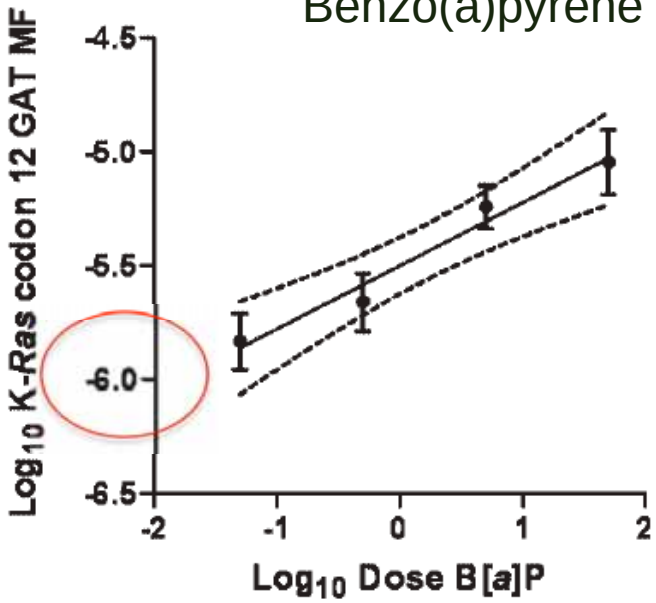
K-Ras Mutations: Comparison with Benzo(a)pyrene (Mouse Duodenum, Day 91)

- K-Ras Codon 12 GAT mutations increased with BaP dose and adduct formation in mouse lung (Meng et al. 2010)
- Evidence for a Mutagenic MOA for BaP in lung
- High background rate of K-Ras mutations in mouse small intestine as compared to lung and other tissues (Mutant fraction of ~10^{-3} in intestine and ~10^{-6} in lung)

Cr(VI)

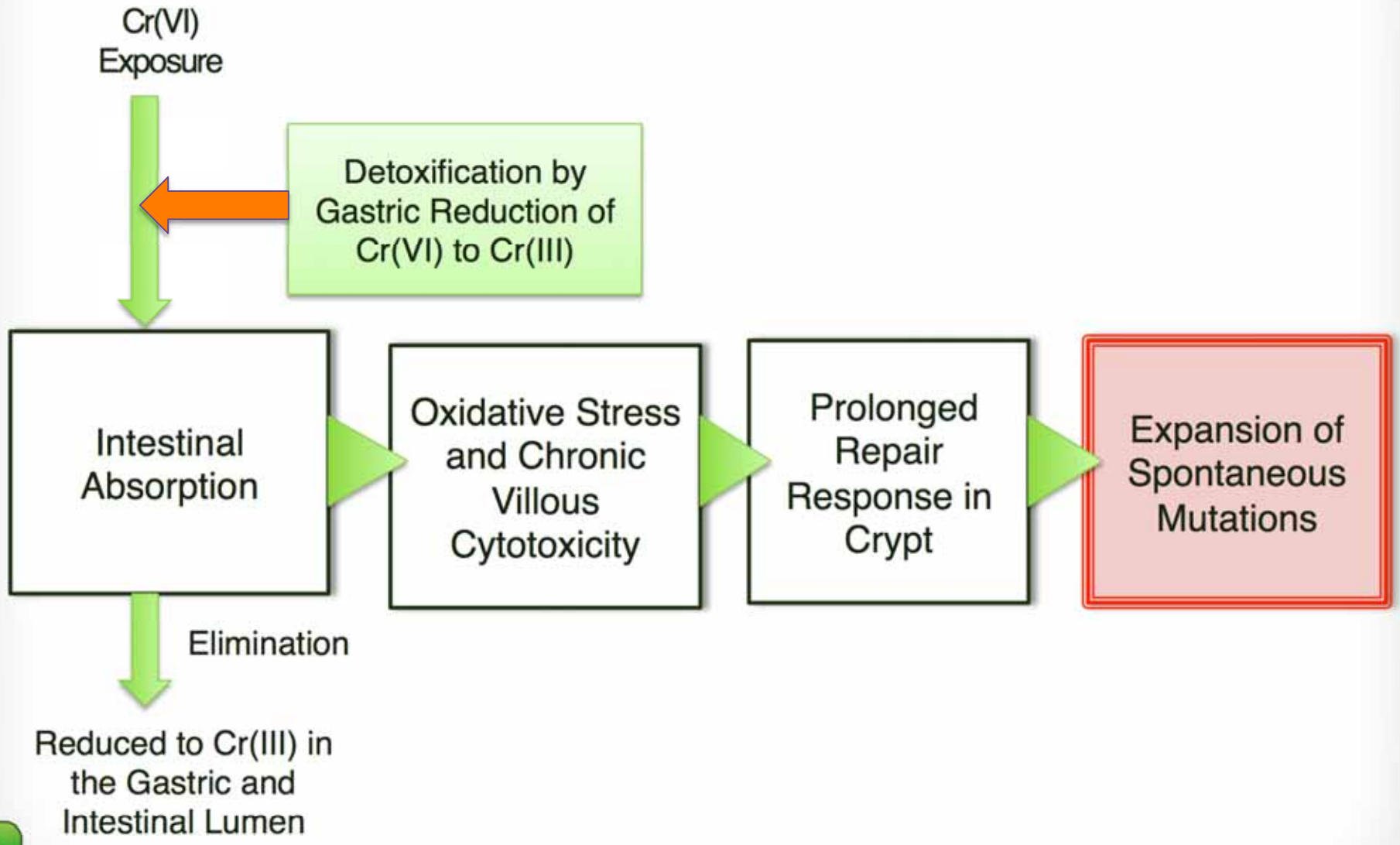


Benzo(a)pyrene



From Meng et al. 2010. *Environ Mol Mutagen* 51, 146-155

Lack of Early DNA Damage or Mutations



MOA Study Findings (Mice)

Significant change	Cr6 Drinking Water Concentration (mg/L)					
Day 91 Duodenum	**0.1**	**1.4**	**5**	**20**	**60**	**180**
Cr in duodenum	-	-	✓	✓	✓	✓
Oxidative Changes	-	-	✓	✓	✓	✓
Gene Changes	-	-	✓	✓	✓	✓
Villus toxicity	-	-	-	✓	✓	✓
Crypt proliferation	-	-	-	-	✓	✓
Crypt DNA damage	-	-	-	-	-	-
K-Ras mutation (Codon 12 GAT)	-	-	-	-	-	-

Underlined checks indicate significant changes at day 8 as well, Cr concentrations not measured at day 8.

MOA Study Findings (Mice)

Significant change	Cr6 Drinking Water Concentration (mg/L)					
Day 91 Duodenum	**0.1**	**1.4**	**5**	**20**	**60**	**180**
Cr in duodenum	**No Effect in the Low Dose Range**		✓	✓	✓	✓
Oxidative Changes			✓	✓	✓	✓
Gene Changes			✓	✓	✓	✓
Villus toxicity			-	✓	✓	✓
Crypt proliferation			-	-	✓	✓
Crypt DNA damage			-	-	-	-
K-Ras mutation (Codon 12 GAT)			-	-	-	-

Underlined checks indicate significant changes at day 8 as well, Cr concentrations not measured at day 8.

MOA Study Findings (Mice)

Significant change	Cr6 Drinking Water Concentration (mg/L)					
Day 91 Duodenum	**0.1**	**1.4**	**5**	**20**	**60**	**180**
Cr in duodenum	-	-	✓	✓	✓	✓
Oxidative Changes	-	-	✓	✓	✓	✓
Gene Changes	-	-	✓	✓	✓	✓
Villus toxicity	-	-	-	✓	✓	✓
Crypt proliferation	-	-	-	-	✓	✓
Crypt DNA damage						
K-Ras mutation (Codon 12 GAT)						

No Mutagenesis, No Basis for Linear Low Dose Extrapolation

Underlined checks indicate significant changes at day 8 as well, Cr concentrations not measured at day 8.

Use of PBPK Models

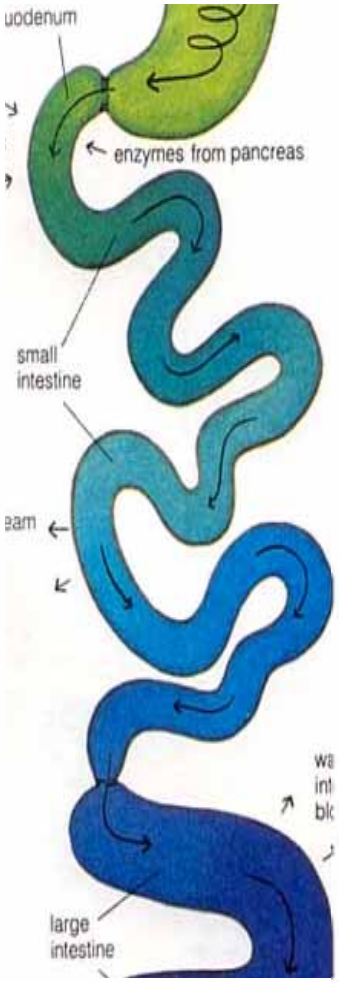
PBPK Models Can Predict Internal Dose by Intestinal Segment

Stomach

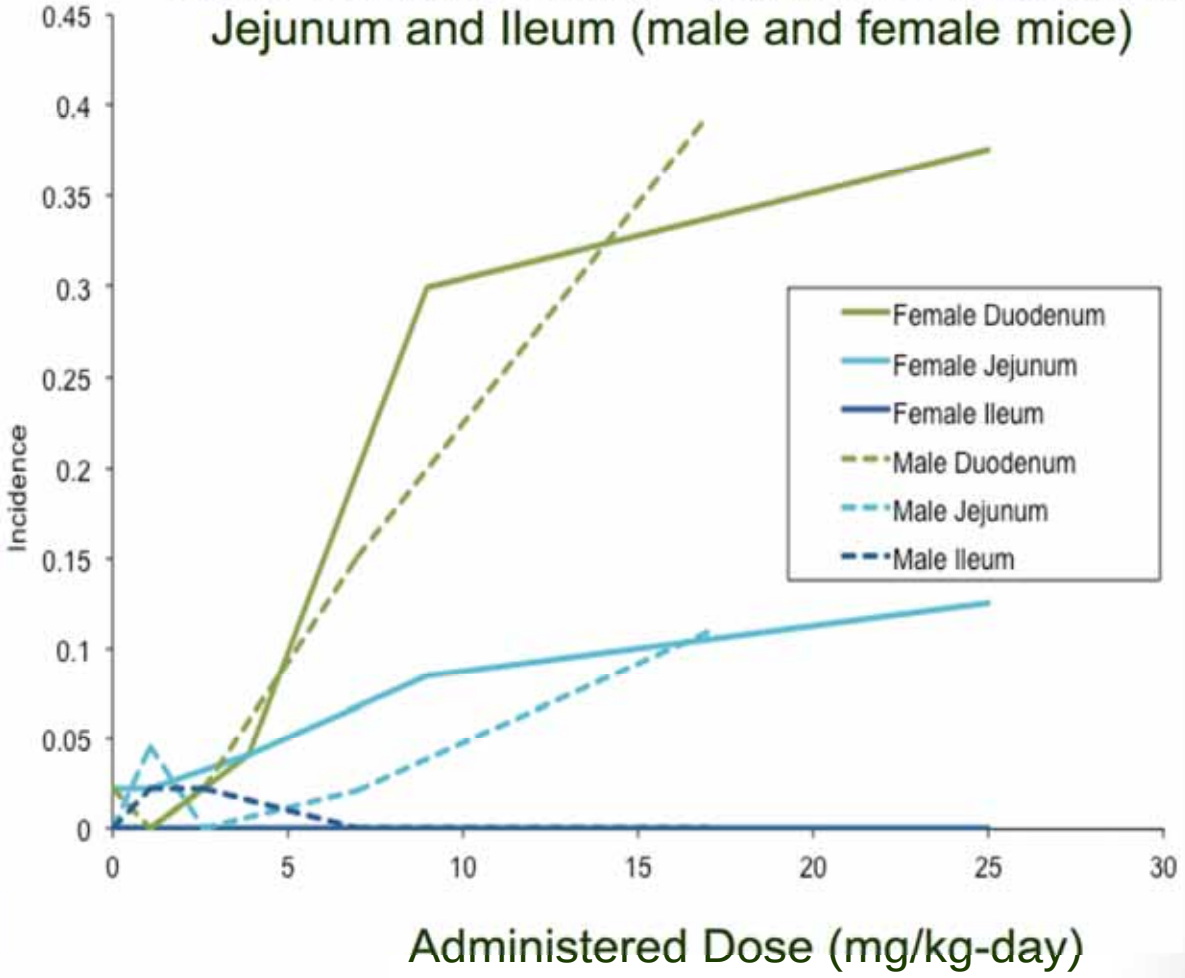
Duodenum

Jejunum

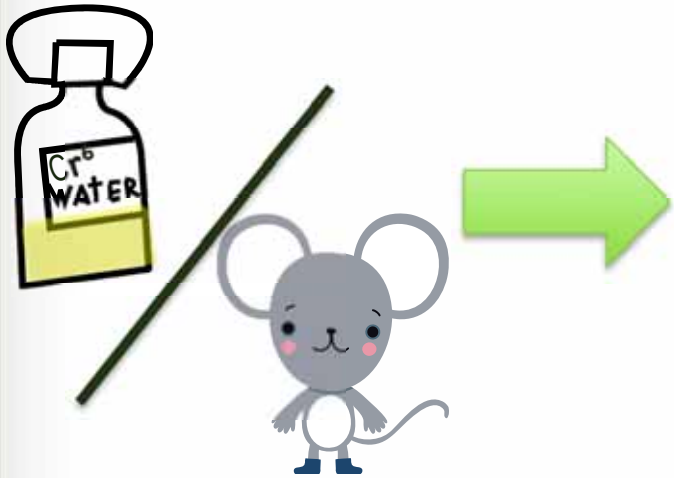
Ileum

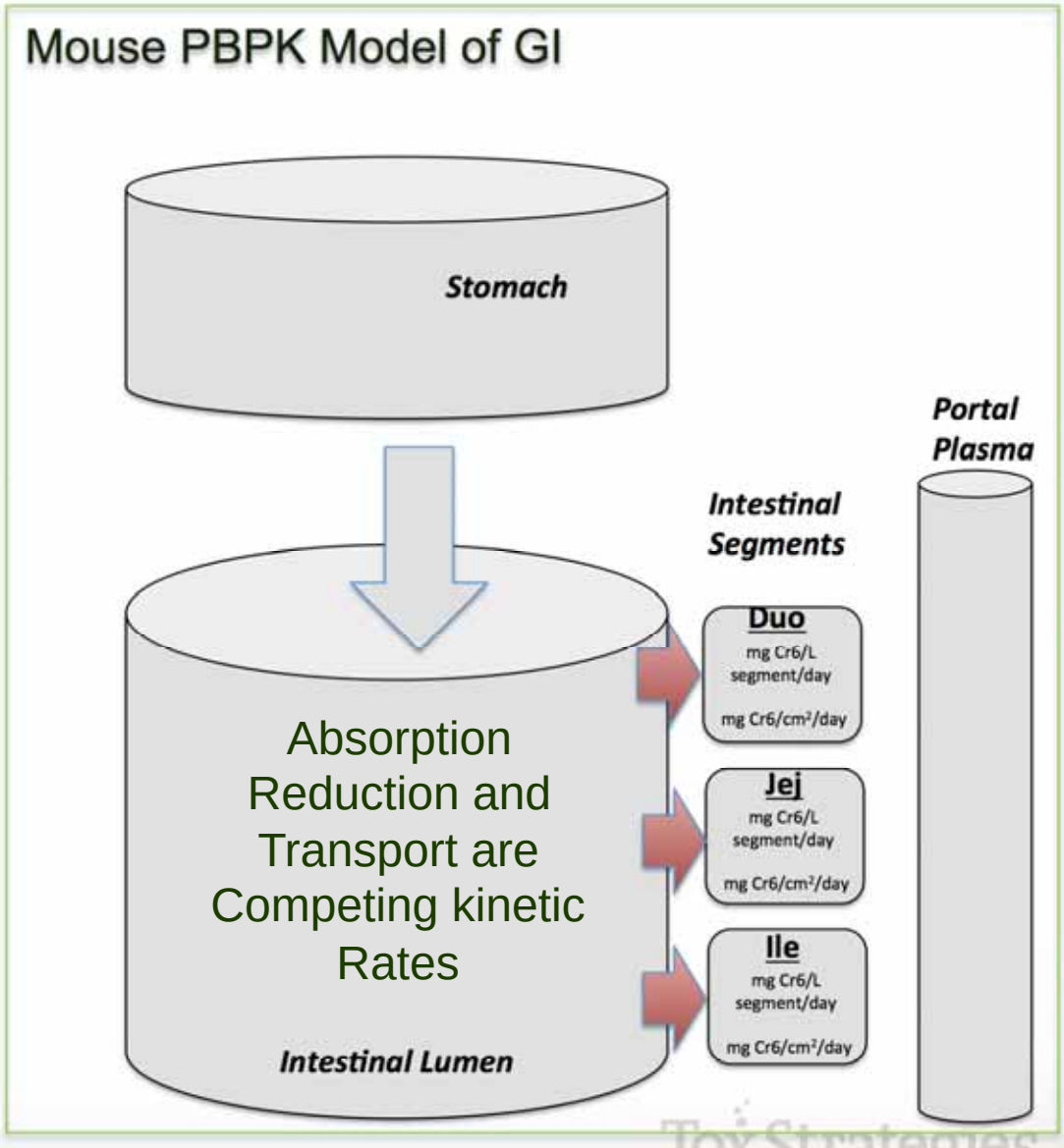


Tumor Incidence in NTP Study in the Duodenum Jejunum and Ileum (male and female mice)



PBPK Mouse Model Allows for Measures of Cr(VI) Absorption into Small Intestine Tissues





Results in a More Robust Dose-Response Data Set!

Traditional Risk Assessment Relies on 6 Dose groups including controls (50 animals per dose) Drinking Water Dose

With PBPK, Drinking Water Dose Can be Converted to Internal Dose

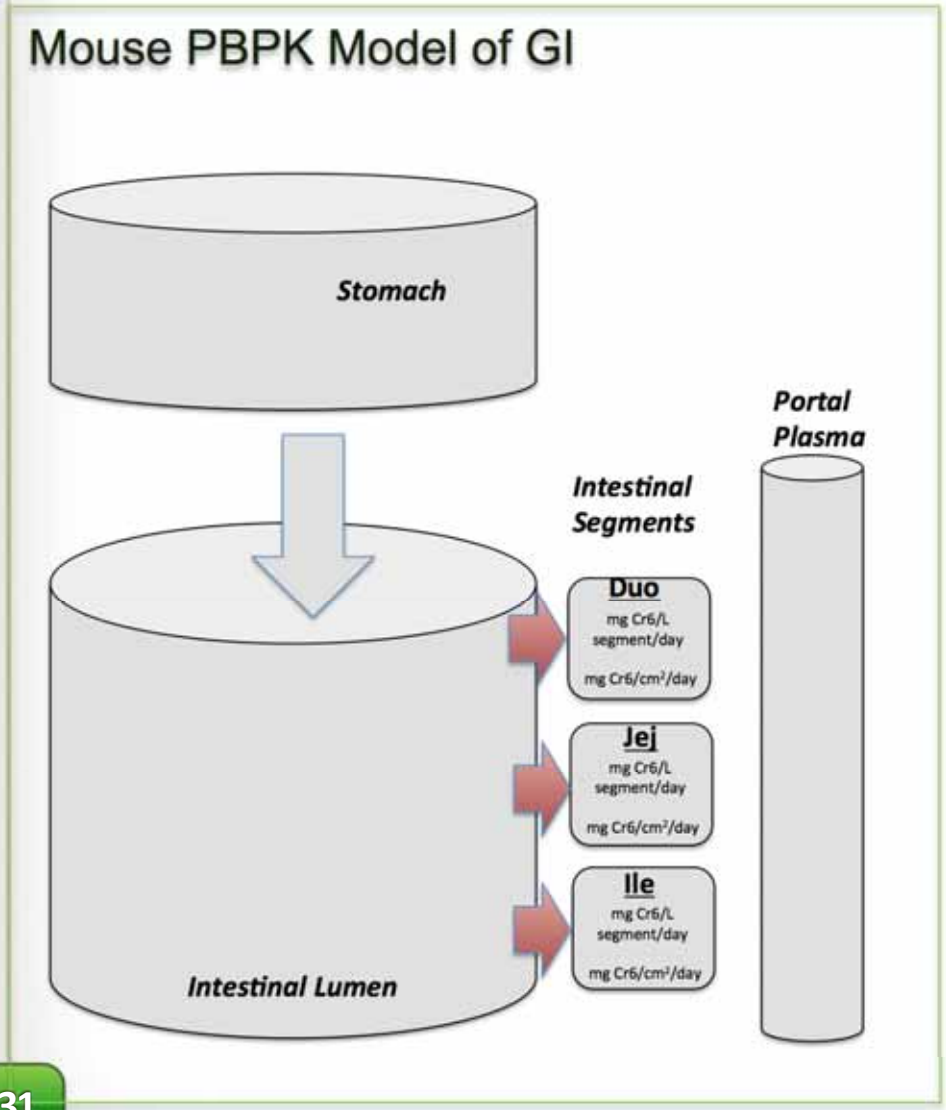
Diff Hyperplasia in M&F Duo, Jej, & Ile

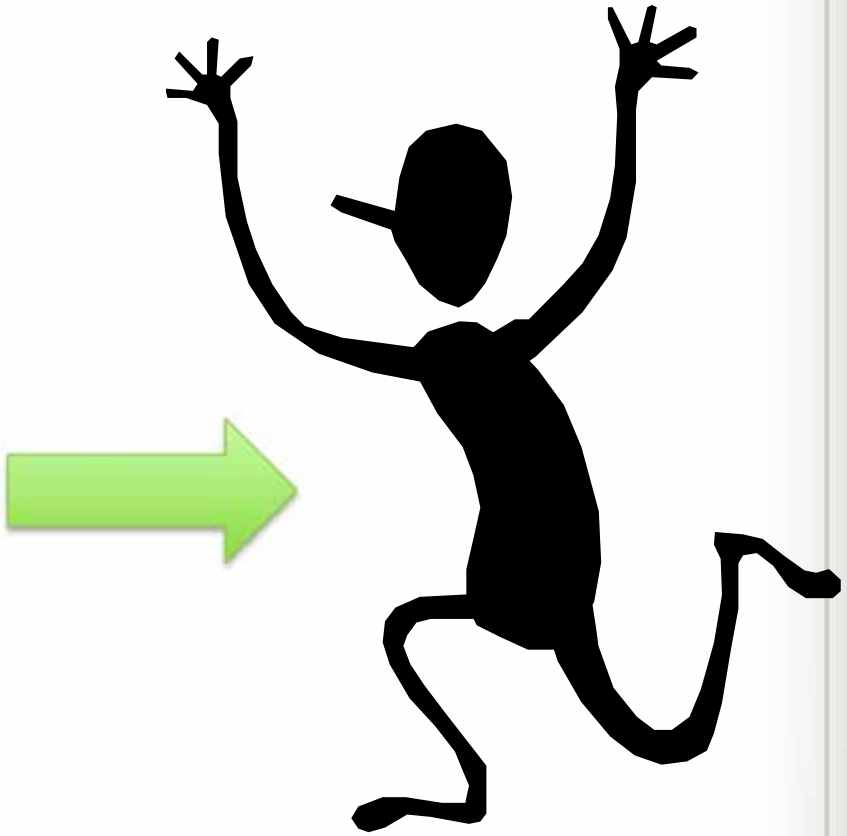
tissue	sex	flux	N	effect
dji	m&f	0	100	0
i	f	0.03	50	0
i	m	0.04	50	0
i	m	0.07	50	0
i				
i				
j				
j				
i				
i				
j				
i				
j				
j				
d				
d				
j				
j				
d				
j				
d	f	7.52	50	35
d	m	10.73	50	42
d	f	15.83	50	31
d	m	20.28	50	32
d	f	28.41	50	42

PBPK Based Risk Assessment Allows for More Effective Use of Same Data Set
(50 animals per dose)

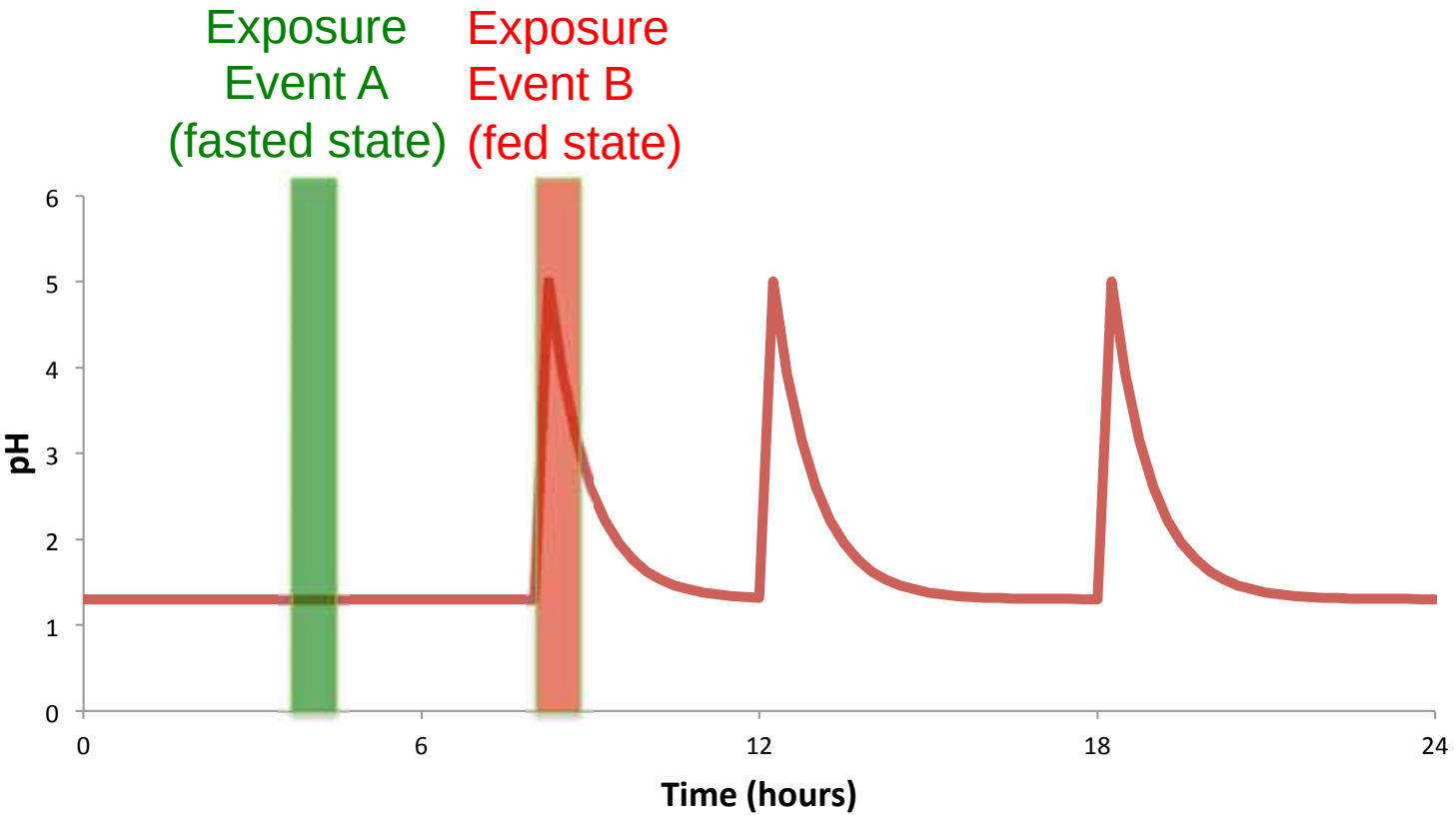
- **Can use males and females**
- **Can use each intestinal segment of each animal**
- **Based on Cr(VI) absorption into target tissue**

Human PBPK Model Allows for Extrapolation of Internal Dose to Humans



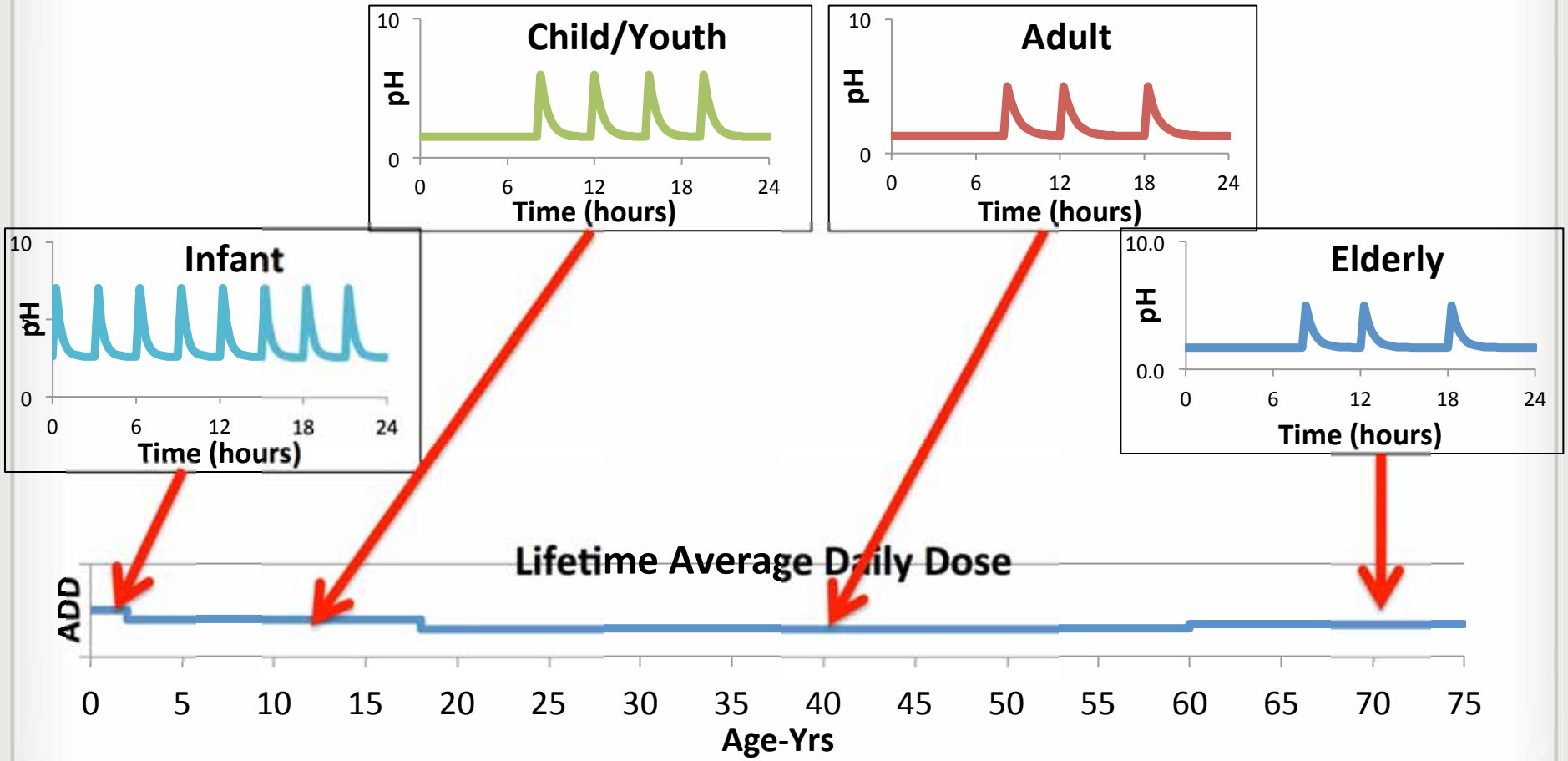


Model Application to Humans: Importance on Exposure Time and Diurnal Variation



Because Cr(VI) reduction is pH-dependent, exposure events A & B will result in different internal doses even if external doses are the same

Model Application for Risk Assessment: Accounting for Age-Dependent Changes in Gastric pH



The lifetime average weighted dose (a time-weighted average) is less than 2-fold greater than the adult average daily dose

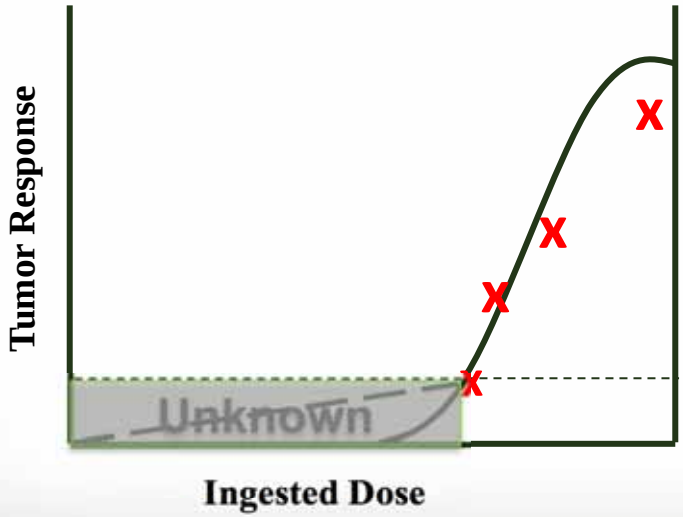
Using Mode of Action and PBPK Models in Risk Assessment

ToxStrategies

EPA's Draft Risk Assessment with a Mutagenic MOA Compared with That Considering The New Data

Mutagenic Sequence of Events (EPA's current draft model)
DNA modification
Cr-Induced Mutation
Cell proliferation
Tumor

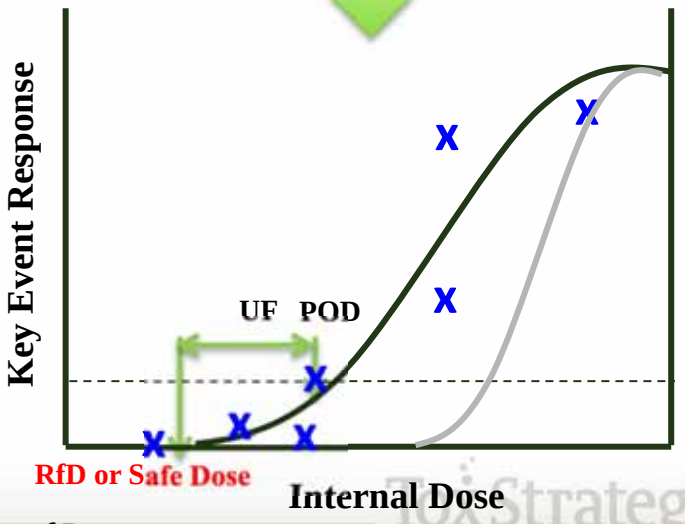
Risk Assessment



Non-mutagenic Sequence of Events (Based on Research Findings)
Saturation of reduction
Villous cytotoxicity
Crypt Cell Proliferation
Spontaneous Mutation
Tumor

PBPK Model

Risk Assessment



UF = Uncertainty Factor, POD = Point of Departure

Risk Assessment Terms

Point of Departure (POD)

Lower confidence level on

10% response level

Uncertainty Factor (UF)

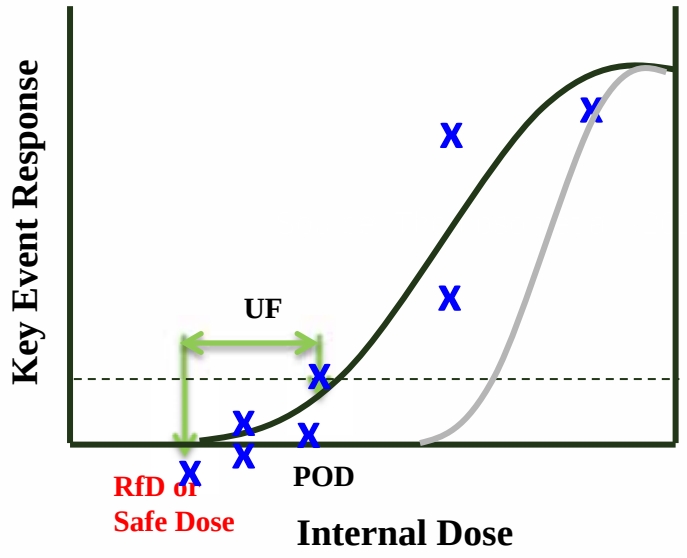
3-10 Multipliers Used to Account

for uncertainty and unaccounted

for variability

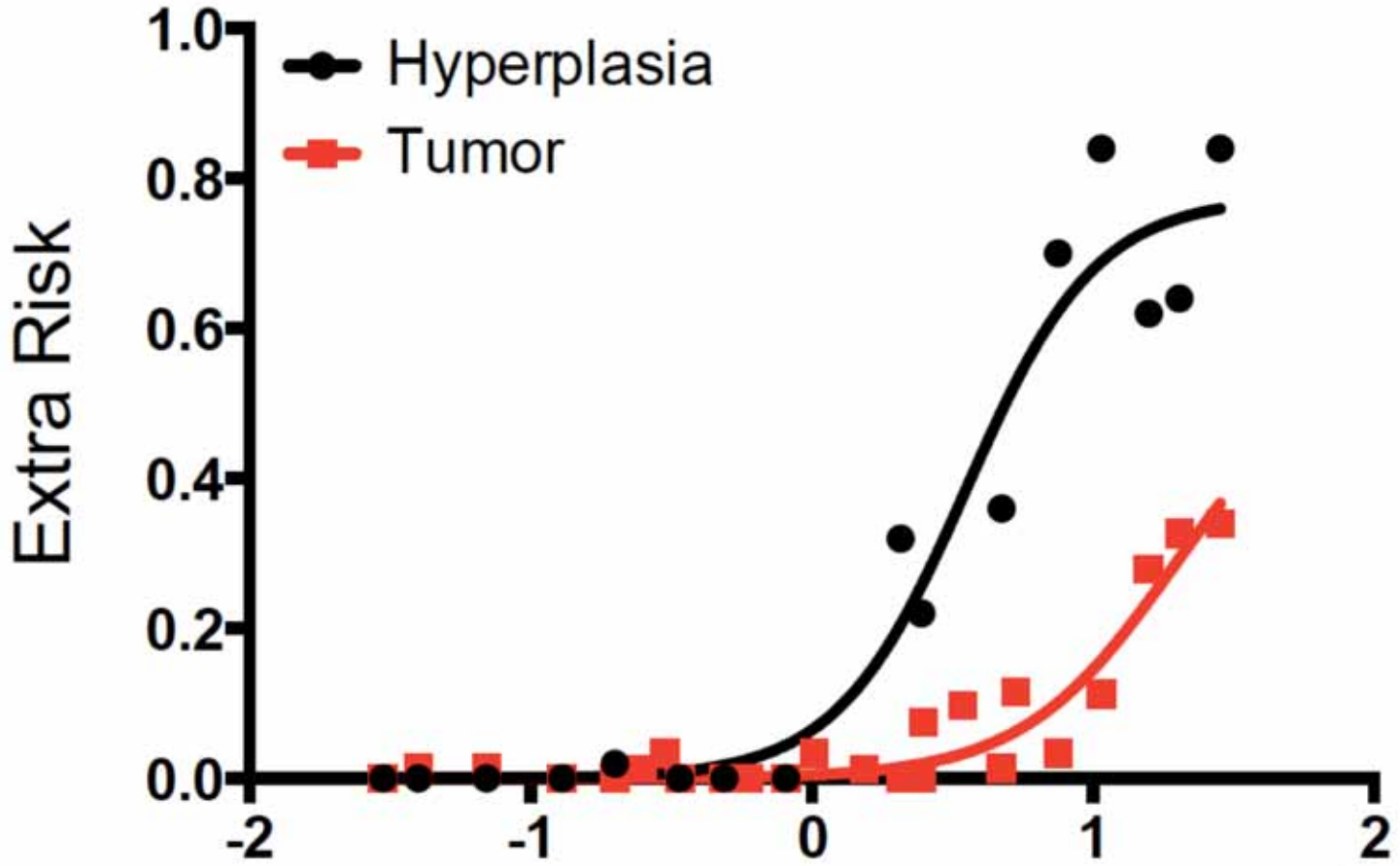
Reference Dose (RfD)

Example Dose Response Curve



An RfD is defined by the U.S. EPA as "*an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime*."

Hyperplasia is Key Event Preceding Cancer (NTP 2-year Data)



Flux (absorption) of Cr(VI) into mouse small intestinal tissue for all three segments of the small Intestine

Calculation of a Reference Dose Protective of Intestinal Cancer

$$RfD = LADD \div UF$$

Accounts for pH variability at all life stages

RfD = Reference Dose (mg/kg-day)

LADD = Lifetime Average Daily Dose (mg/kg-day) in Human

UF = Uncertainty Factors

People on Proton Pump Inhibitors have ~3-fold higher dose
* Variations in Water Consumption can result in up to a higher dose by ~2-fold

RfD Can Be used To Calculate a DWEL

Drinking Water Equivalent Level (DWEL) = (RfD x BW) ÷ IR

DWEL = Drinking Water Equivalent Level

RfD = Reference Dose for a Lifetime exposure including sensitive subpopulations

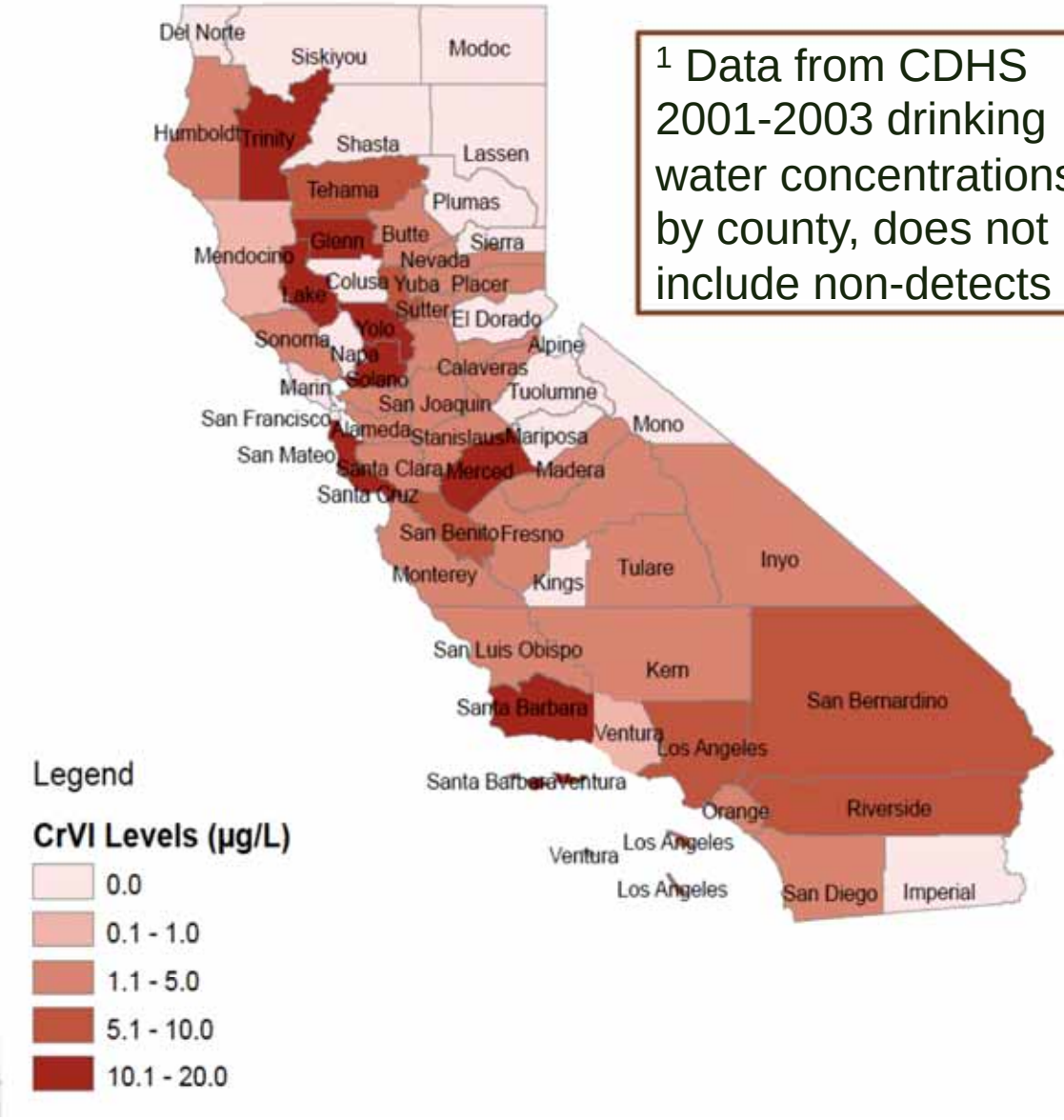
IR = Ingestion Rate (2 L/day)

BW = Body Weight (70 kg)

Resulting DWEL is supportive of the current MCL (~100 ppb)

Average Cr(VI) Detected in Drinking Water[1]

- Background is around 1-5 ppb
- DWEL is higher than background
- Current Standards are protective
- No risk at normal background exposures, even for sensitive subpopulations



[1] Data from CDHS 2001-2003 drinking water concentrations by county, does not include non-detects

Summary Conclusion

- Research provides strong support for a cytotoxic MOA
  - At non-cytotoxic doses, this MOA is not operable
- This MOA is consistent with the notion that there is an exposure level that does not pose an increased cancer risk
- PBPK models are needed for risk assessment
  - Differences in internal dose between species (mice and humans)
  - Extrapolation between the high doses that caused tumors in rodents and environmentally relevant drinking water exposures
- DWEL is consistent with current Drinking Water Standards

Questions and Discussion