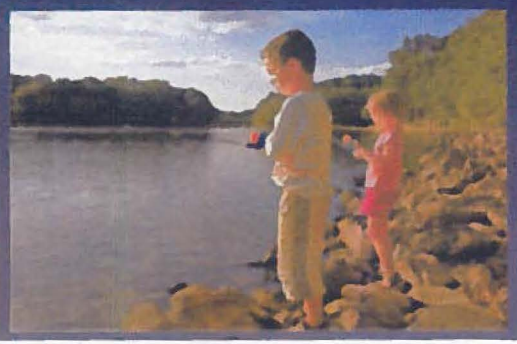
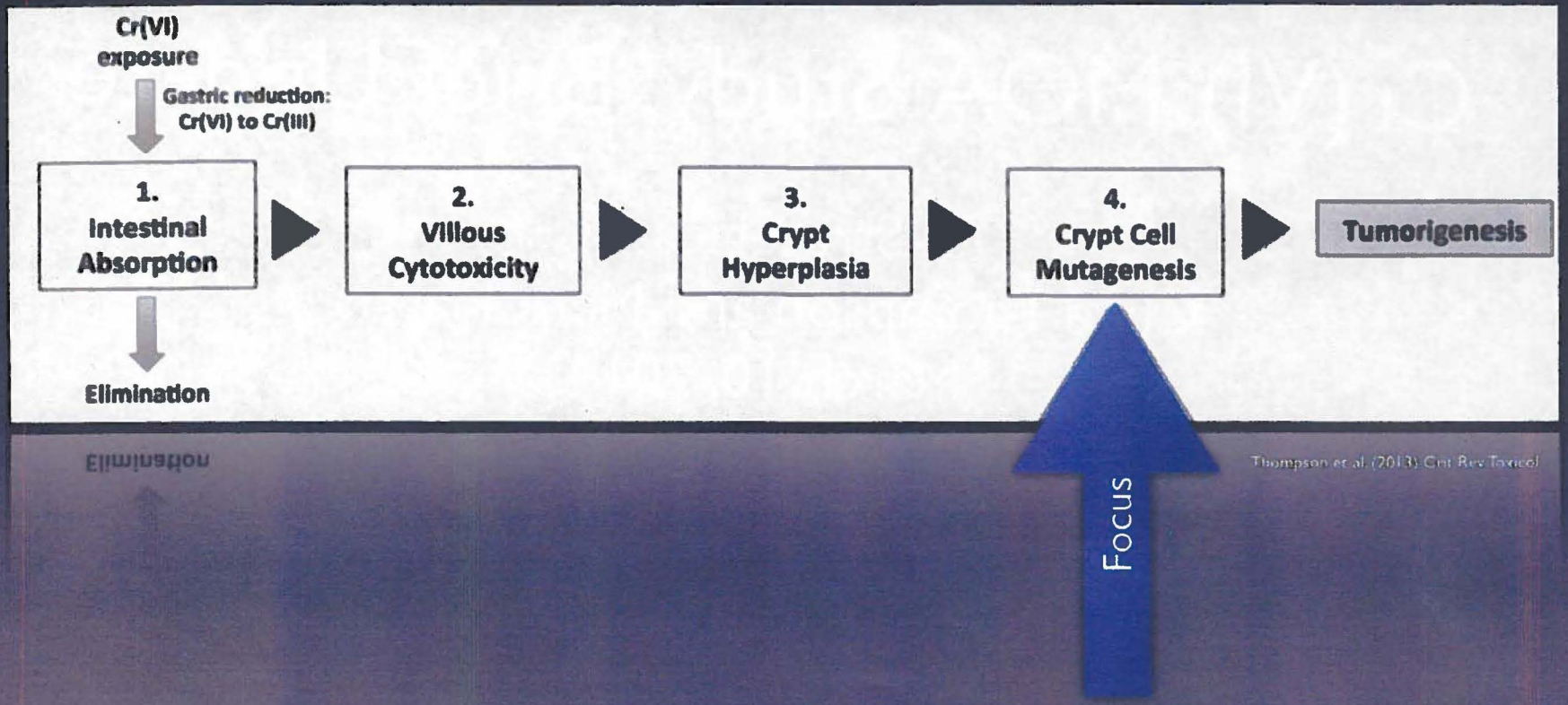
Cr(VI) MOA Study: Brief Update

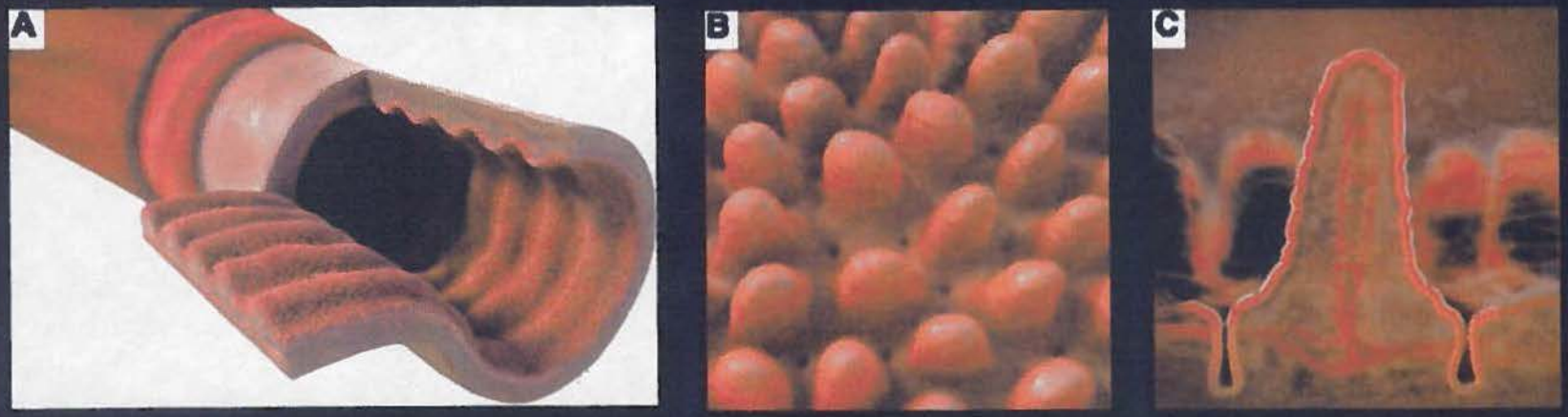
ToxStrategies, Inc.
Chad Thompson and Mark Harris
March 10, 2014

MOA for Cr(VI)-Induced Intestinal Carcinogenesis



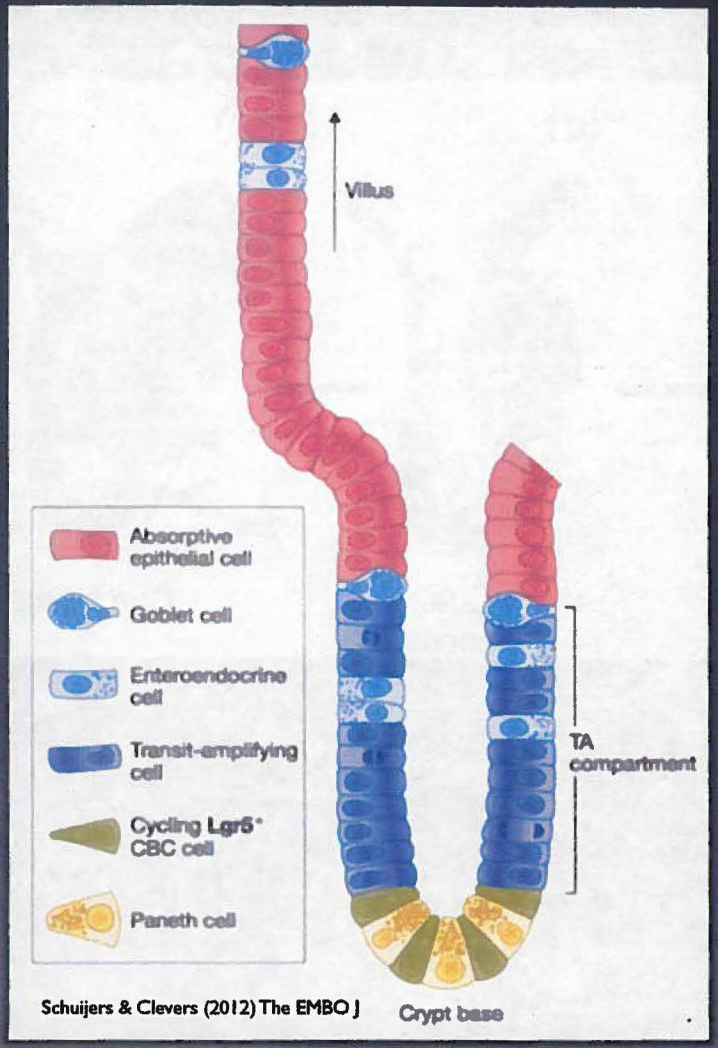
Structure of Small Intestine



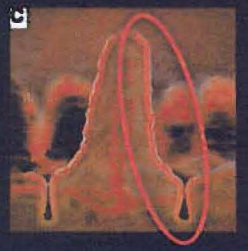
Schepers & Clevers (2012 The EMBO J

Crypt-Villus Unit

Stem Cells Reside at the Crypt Base

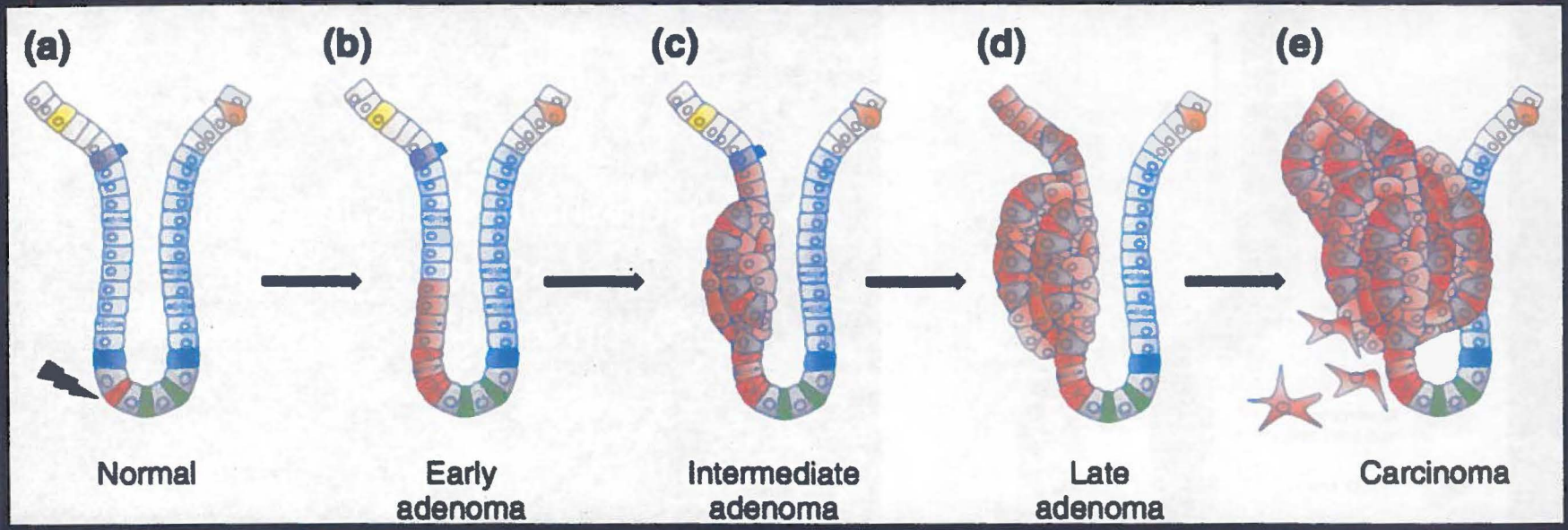


Schuijers & Clevers (2012) The EMBO J



- TA epithelial cells leave crypt, differentiate, and become absorptive villous enterocytes
- Transit amplifying cells give rise to absorptive and non-absorptive cells
- Stem cells reside at base of crypt

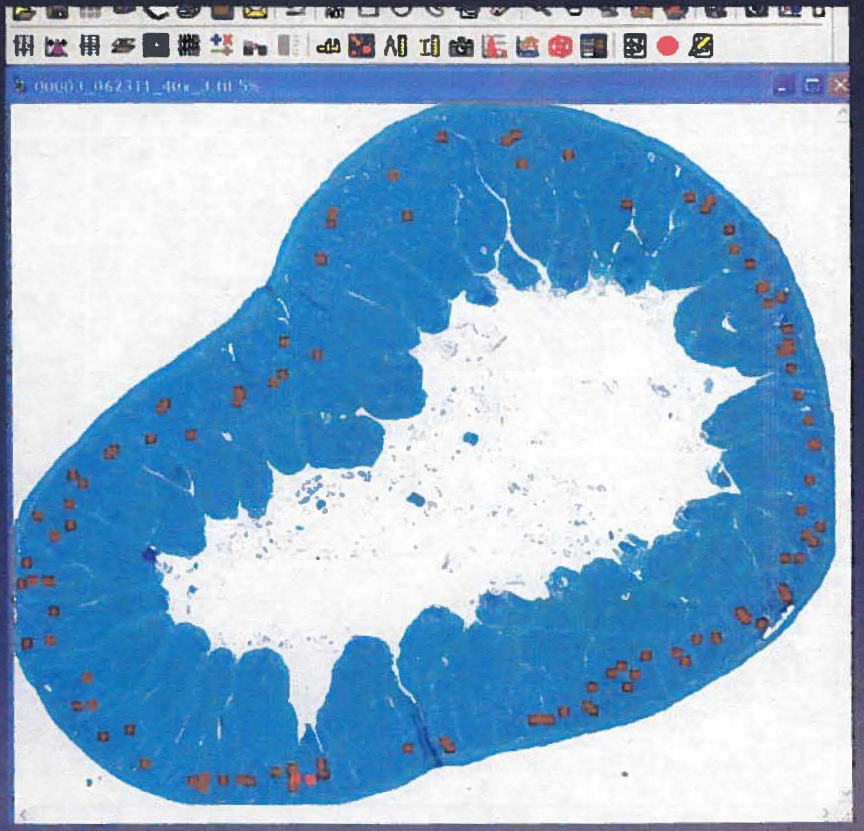
Model of Intestinal Carcinogenesis



Ryde & Barker (2012) WIREs Systems Biology and Medicine

In Vivo Micronucleus Assay

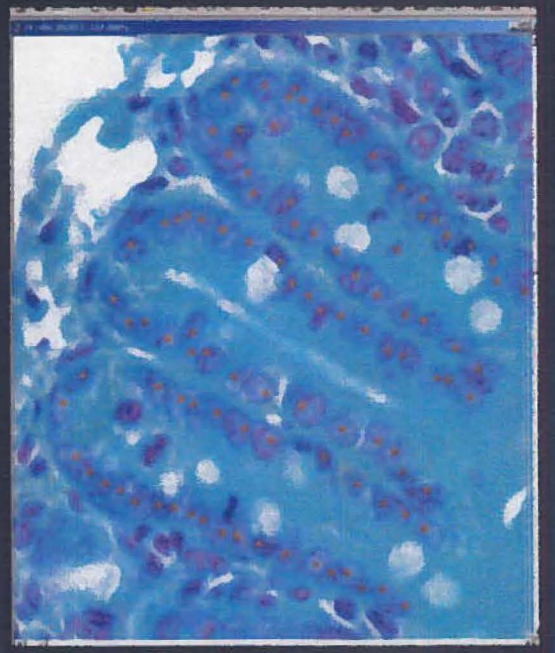
"This mammalian *in vivo* micronucleus test is especially relevant to assessing mutagenic hazard...An in vivo assay is also useful for further investigation of a mutagenic effect detected by an in vitro system." (OECD, Guideline Study 474)



No Micronuclei in Fully Intact Crypts

mg/L SDD	Enterocytes	MN
0	1921	0
0.3	1707	0
5	1825	0
14	1420	0
60	2386	0
170	2746	0
520	3194	0

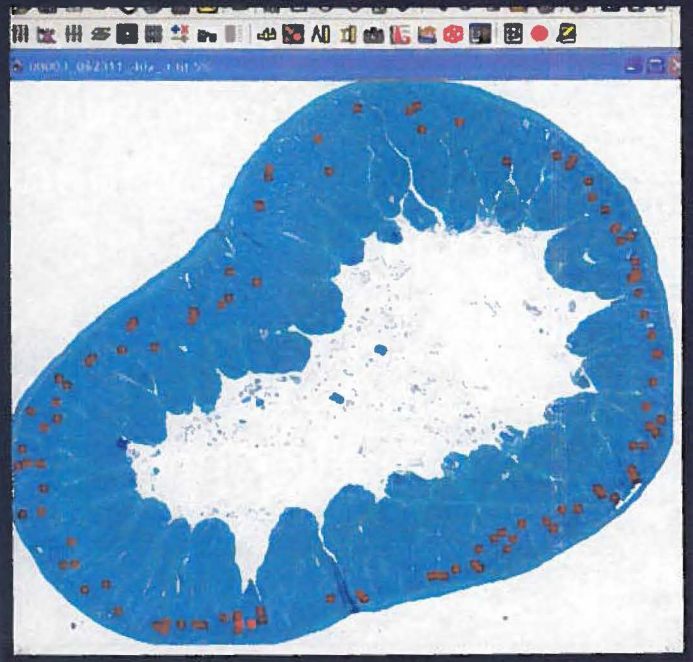
10 fully intact crypts per animal (4-5 animals/dose)



Micronuclei Across 15 Slides/Group

mg/L SDD	Day 8	Day 91
0	1	2
0.3	0	2
5	0	1
14	0	1
60	0	0
170	0	0
520	0	0

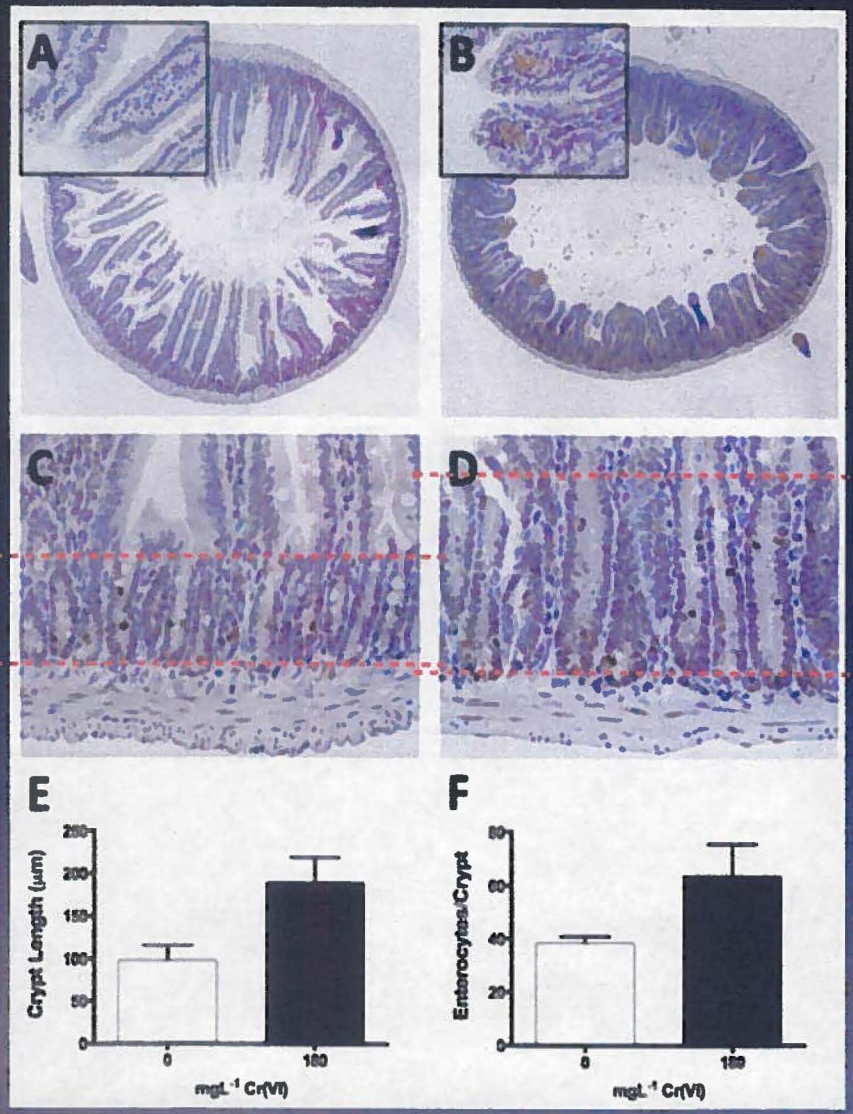
~15 sections (3 slides: 4-5 animals/dose)



Assessment of DNA Damage via γ-H2AX Immunostaining

- DNA double strand breakage results in phosphorylation of histone H2AX
- increased γ-H2AX is a marker of increased DNA damage
- staining in duodena of mice exposed to 520 mg/L SDD was increased in villi, but not crypt

γ-H2AX Not Increased in Crypts

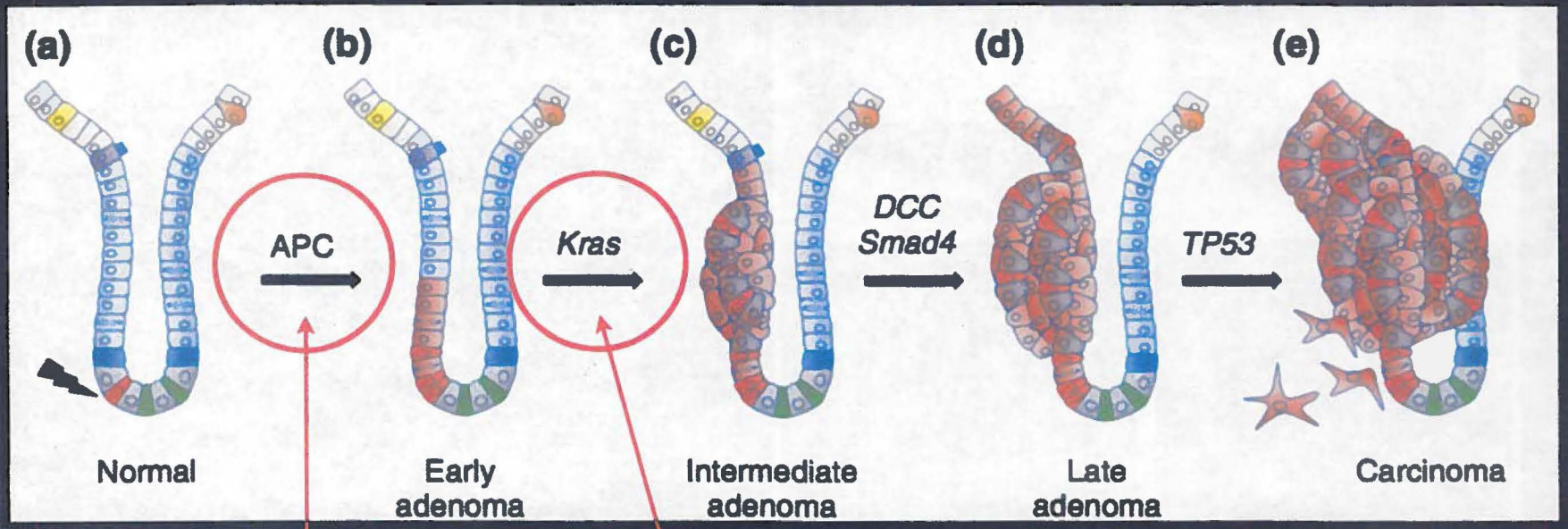


Scoring of γ-H2AX Staining

SDD (mg/L)	Crypt	Villus	Lamina propria
0	2	0	0
0	2	0	0
0	2	0	0
0	2	0	0
0	2	0	0
520	2	1	2
520	2	0	2
520	2	1	2
520	2	1	2
520	2	2	2

Unpublished data

Model of Intestinal Carcinogenesis

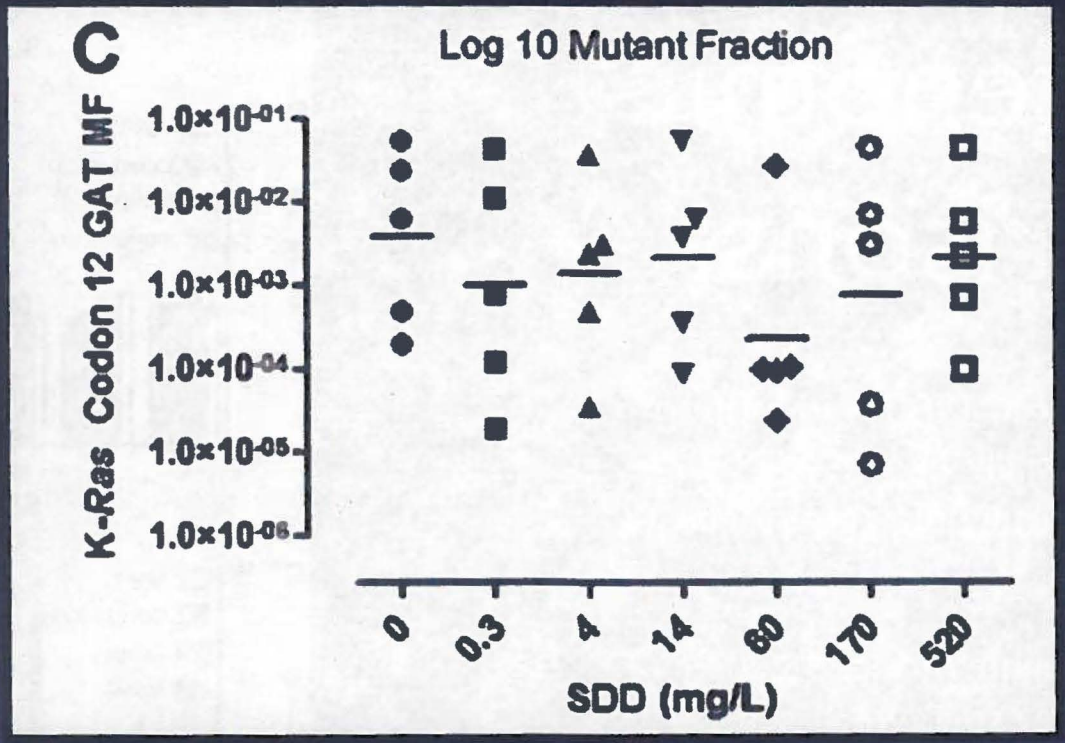


Rios & Barker (2012) WIREs Systems Biology and Medicine

- *Kras* is an early mutation
- have sensitive mutation assay

TF analysis did not indicate activation in APC/β-catenin signaling

ACB-PCR Analysis of *Kras* Codon 12 GAT

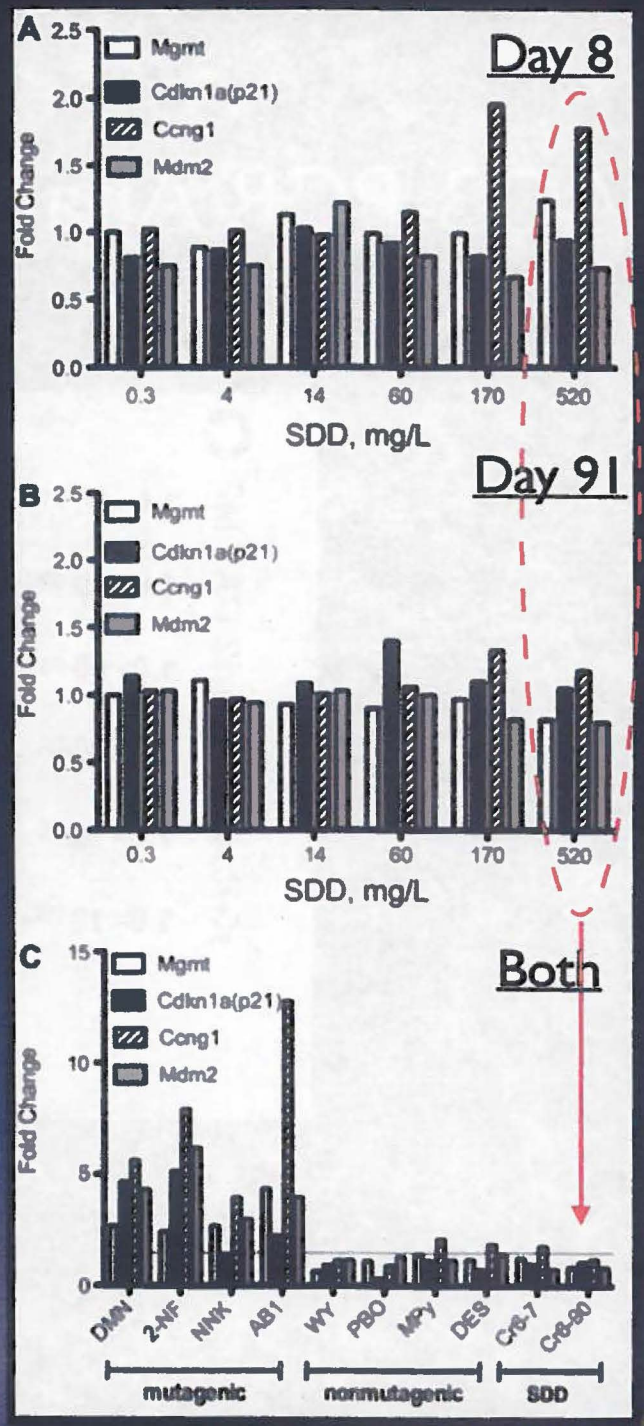


O'Brien et al. (2013) Mut Res

Toxicogenomic Comparisons:

Ellinger-Ziegelbauer et al. (2005) identified several genes differentially expressed by mutagenic and non-mutagenic carcinogens

Several of the most highly induced genes following mutagen exposure were not elevated after either 7 or 90 days of exposure



Thompson et al. (2012) Regul Toxicol Pharm

Summary of Genotoxicity Data

- Cr(VI) induces cytoplasmic vacuolization in villi, but not crypts

- Despite proliferation activity in crypt, no increase in crypt micronuclei (MN)

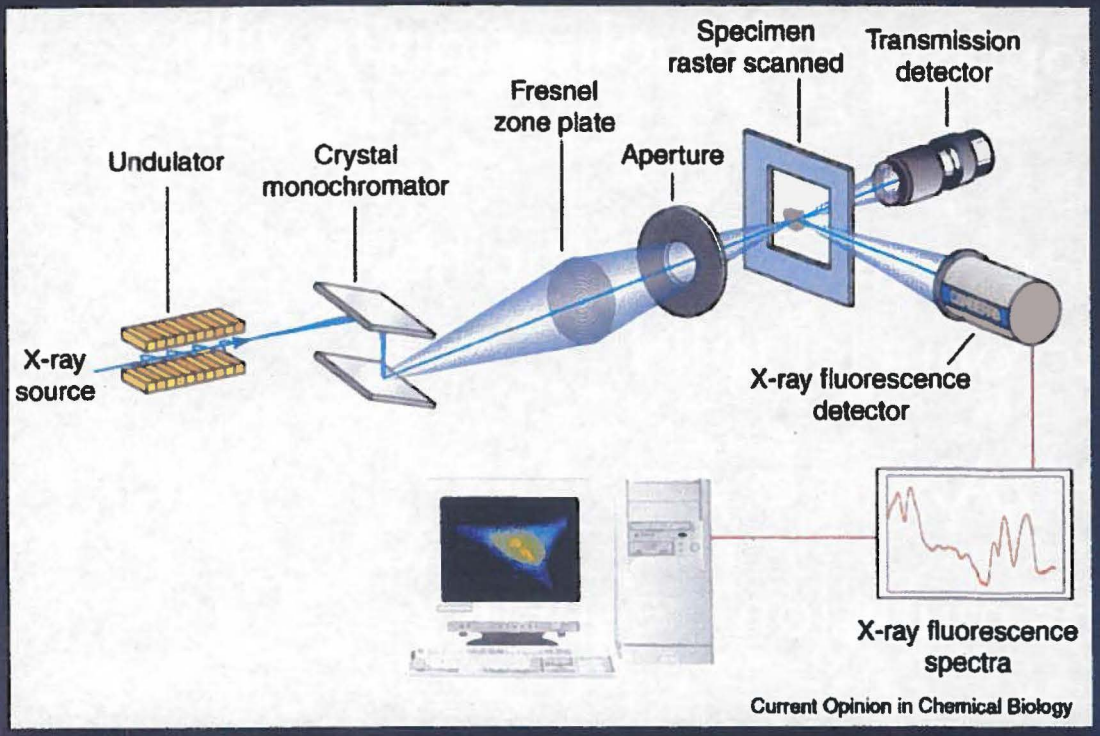
- No increase in γ-H2AX in crypts

- No increase in *Kras* mutation frequency

- Toxicogenomic data indicate changes more consistent with nonmutagenic carcinogens

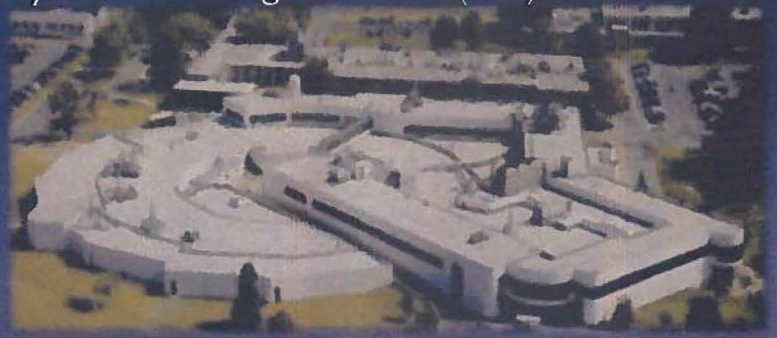
Data suggest Cr(VI) does not reach the crypts

We Can Now Visualize Cr in the Intestine
(using X-ray Fluorescence Microspectroscopy)



Collaborating with US Army Corps of Engineers - Engineer Research and Development Center (ERDC), Vicksburg, MS.

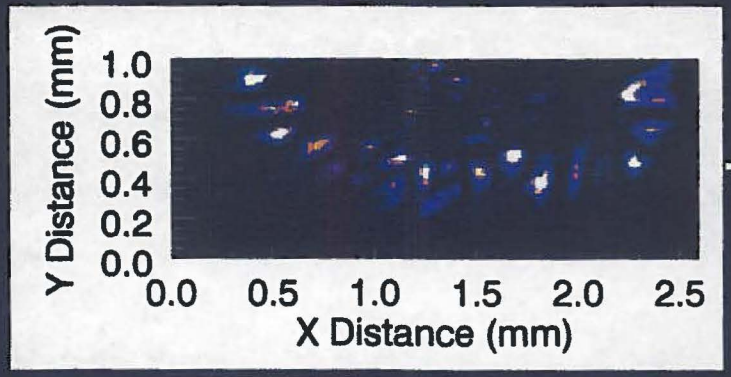
Synchrotron Light Source (SLS):



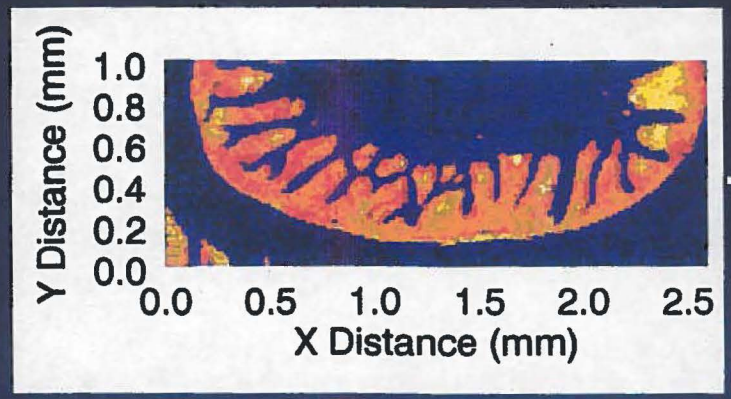
Brookhaven National Lab (Long Island, NY)

Unstained Duodenal Tissue Section
(mouse exposed to 520 mg/L SDD 90 days)

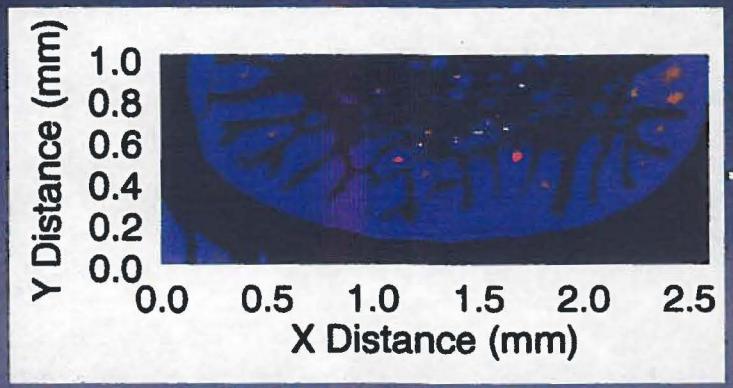
XRF Maps of Elements in Duodenal Sections:



-Cr



-S



-Ca

Unpublished data

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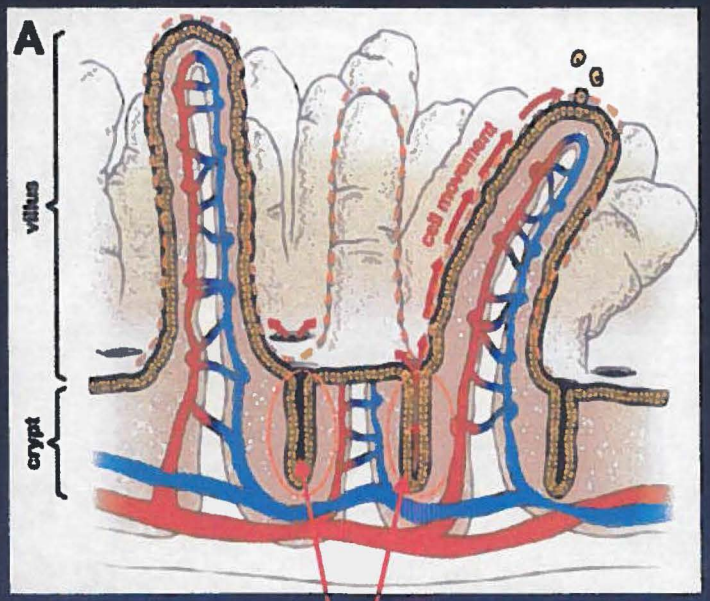
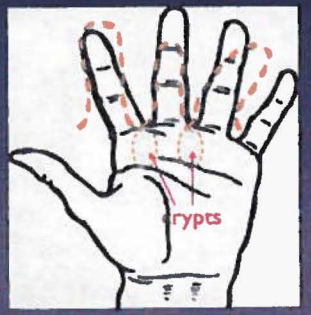
Locating Crypts in XRF Maps

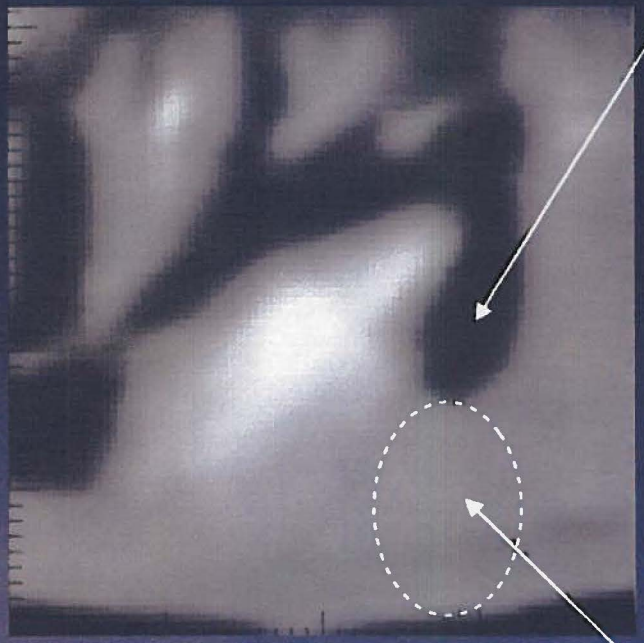


crypts



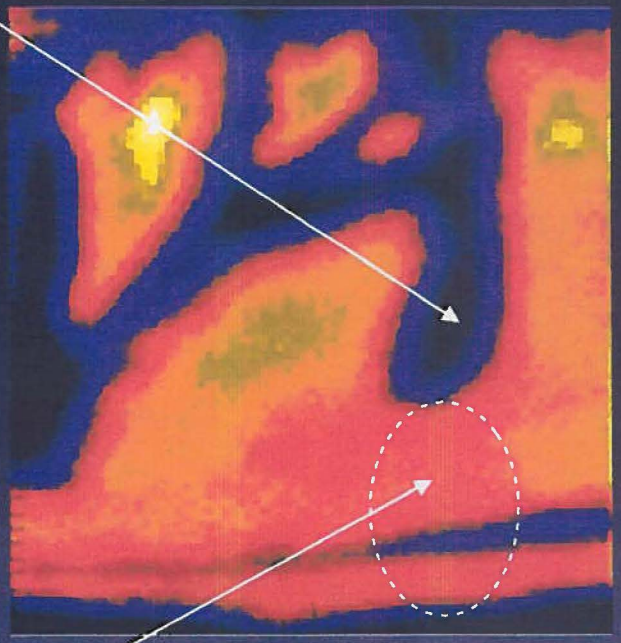
Ca XRF Minimap from Mouse Exposed to 520 mg/L SDD for 90 days

Physical Sample



space b/n 2 villi

Ca XRF Map

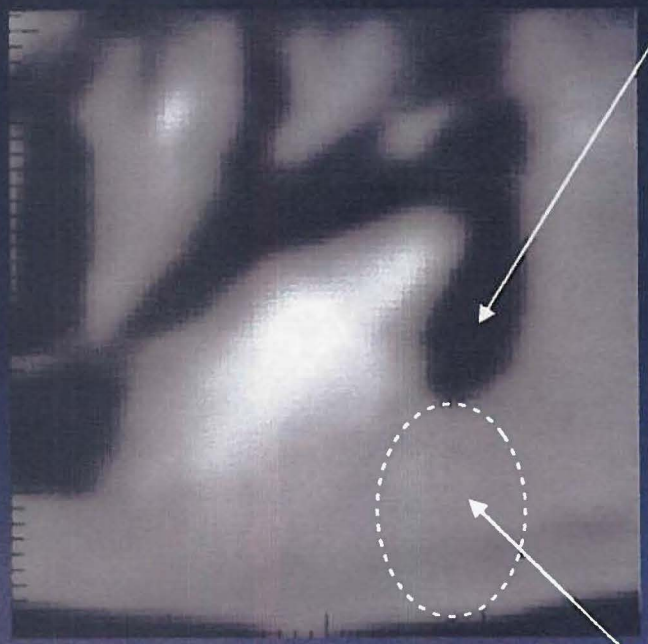


crypt

Unpublished data

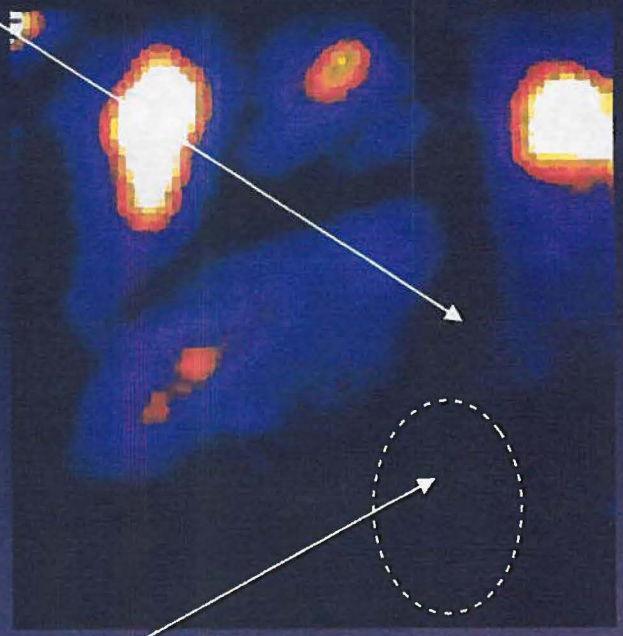
Cr XRF Minimap from Mouse Exposed to 520 mg/L SDD for 90 days

Physical Sample



space b/n
2 villi

Cr XRF Map



crypt

Unpublished data

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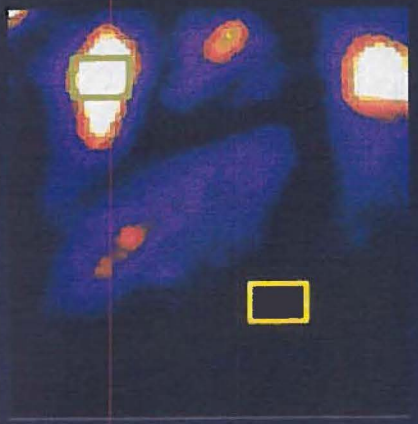
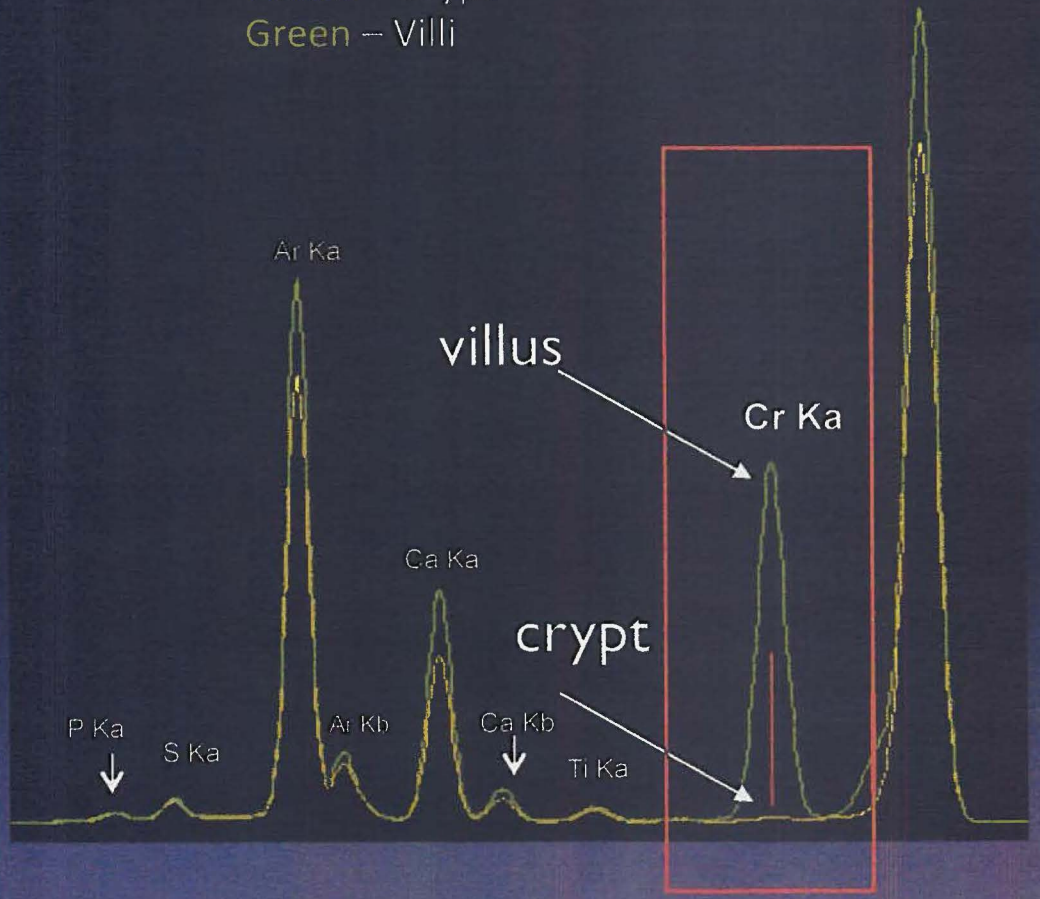
Quantitative Analysis of Crypt & Villi



Yellow – Crypt
Green – Villi



Unpublished data

Cr(VI) MOA Data Summary

	Drinking Water (mg/L SDD)					
	0.3	5	14	60	170	520
Cr in duodenum	–	–	✔	✔	✔	✔
Oxidative changes	–	–	✔	✔	✔	✔
Gene changes	–	–	✔	✔	✔	✔
Villus toxicity	–	–	–	✔	✔	✔
Crypt proliferation	–	–	–	–	✔	✔
Crypt MN	–	–	–	–	–	–
Kras mutation	–	–	–	–	–	–
γ-H2AX	–	–	–	–	–	–

Underlined checks indicate significant changes at day 8 as well. Note Cr was not measured at day 8.

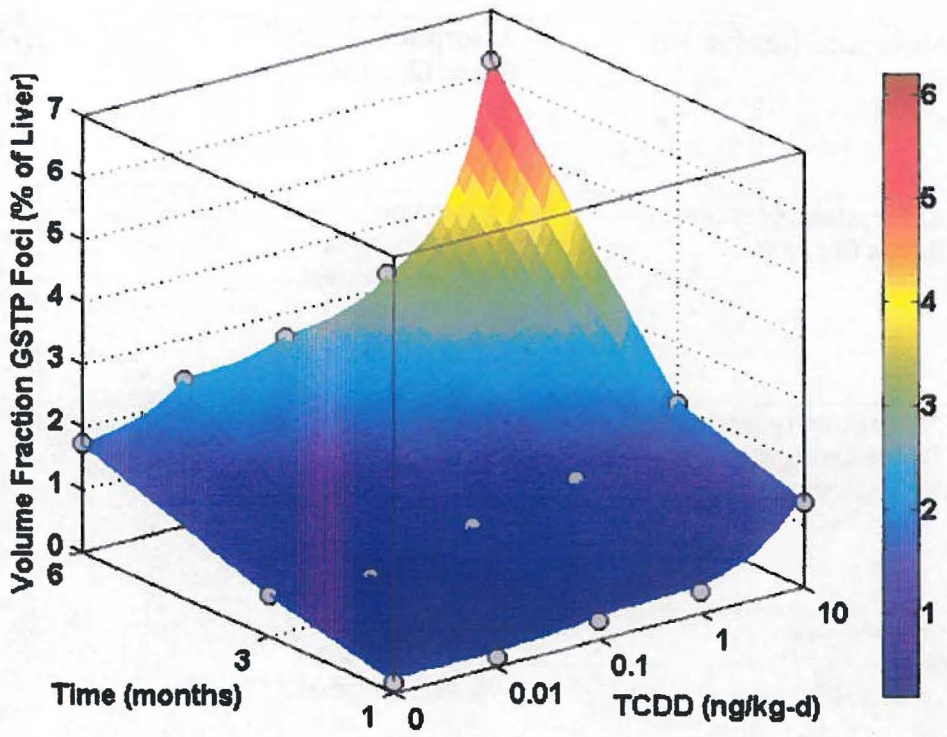
Summary

- MOA research suggests nonmutagenic MOA
- Synchrotron-based research suggest that little or no Cr can be detected in the crypts of mice exposed to 180 mg/L Cr(VI)
  - consistent with lack of crypt genotoxicity
  - supports proliferation based MOA
  - lower water Cr concentrations *highly* unlikely to reach crypts
- Supports the use of nonlinear, RfD-based approach for cancer assessment (similar to captan/folpet; Cohen et al. 2010; FRN, 2004)
- Current drinking water standards should be protective against cytotoxicity and carcinogenicity in the small intestine

Dose-Time Concordance

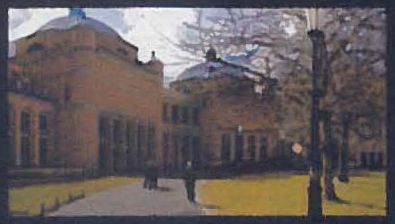
Table 3—Dose-Time Concordance—Page 1				
Time		**8 days**	**90 days**	**720 days**
Increasing Dose (mg/L in drinking water) ↓	**Increasing Time** →		**Duodenum**	
	4		Absorption	No data
	14	Absorption (presumed)	Absorption Redox Changes	Absorption Redox Changes (presumed) Villous Cytotoxicity Crypt Proliferation
	60	Absorption (presumed) Redox Changes	Absorption Redox Changes Villous Cytotoxicity	Absorption Redox Changes (presumed) Villous Cytotoxicity Crypt Proliferation Tumors (historical control)
	170	Absorption (presumed) Redox Changes Villous Cytotoxicity	Absorption Redox Changes Villous Cytotoxicity Crypt Proliferation	Absorption Redox Changes (presumed) Villous Cytotoxicity Crypt Proliferation Tumors (concurrent control)
	520	Absorption Redox Changes Villous Cytotoxicity Crypt Proliferation	Absorption Redox Changes Villous Cytotoxicity Crypt Proliferation	Absorption Redox Changes (presumed) Villous Cytotoxicity Crypt Proliferation Tumors (concurrent control)

3D Graphs in Dose and Time



Acknowledgements

Universities



Michigan State University
Duke Univ. Medical School
Univ. of Cincinnati Medical Center
George Washington Univ. Med. Center

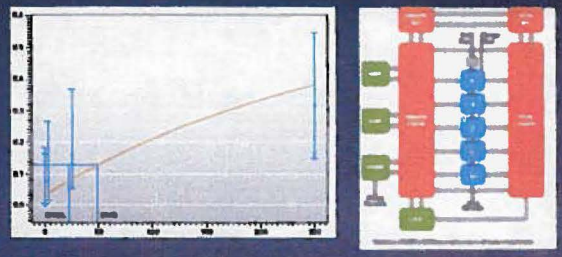
Research Laboratories

Southern Research Institute
Experimental Pathology Laboratories
National Center for Toxicological Research
U.S. Army Engineer Research & Development Center

Analytical Laboratories

ThermoFisher
Applied Speciation
Brooks Rand Laboratory
Environmental Standards

Risk Assessors



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Summit Toxicology

This research was funded by the American Chemistry Council

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Publications

Topic	Publication
Hypothesized MOA	2011, *Toxicol Sci* 119(1)
Mouse 90-day study	2011, *Toxicol Sci* 123(1)
Rat 90-day study	2012, *Toxicol Sci* 125(1)
Mouse genomics	*2012, Toxicol Applied Pharm* 259
Rat genomics	*2012, Toxicol Applied Pharm* 262
Toxicogenomics (PCA)	2012, *Reg Tox Pharm* 64 (1)
In vitro Toxicology	2012, *PLoS ONE* 7(8)
Ex vivo reduction	2012, *Chemosphere* 89
PBPK- Rodents	2012, CBI 200
PBPK- Humans	2013, CBI 204
MOA	2013, Crit Rev Toxicol 43 (3)
Kras Mutation/Cytogenetics	2013, Mut Res 754
Risk Assessment	2013, J Appl Toxicol in press (online now)
Iron Homeostasis	2014, Food & Chem Tox 65