Science Question 4: Mechanistic Studies Database & MOA

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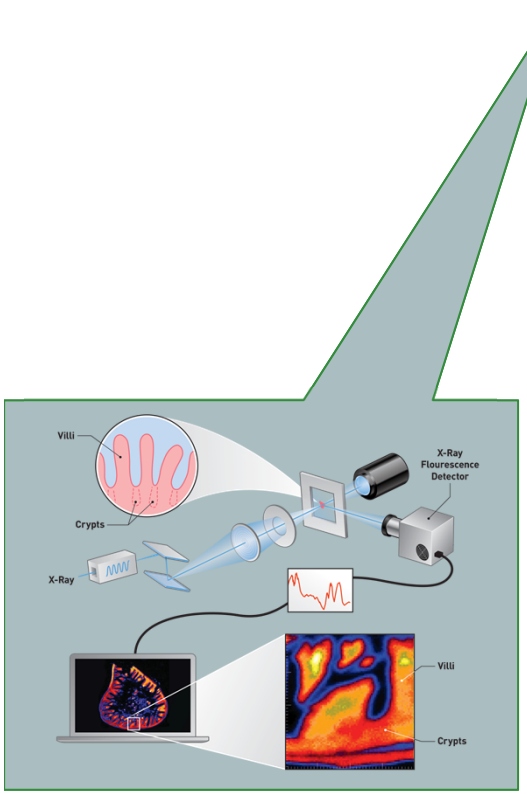
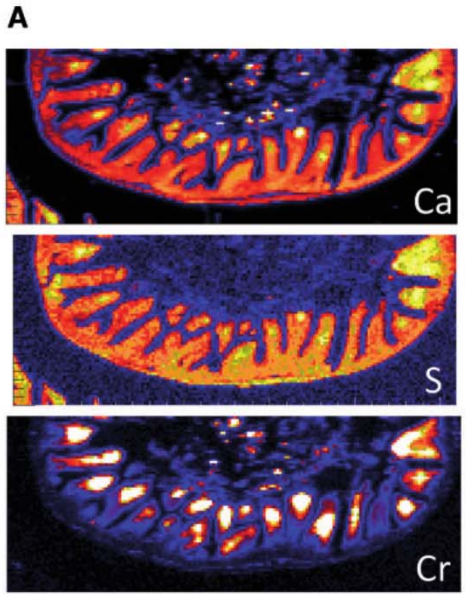
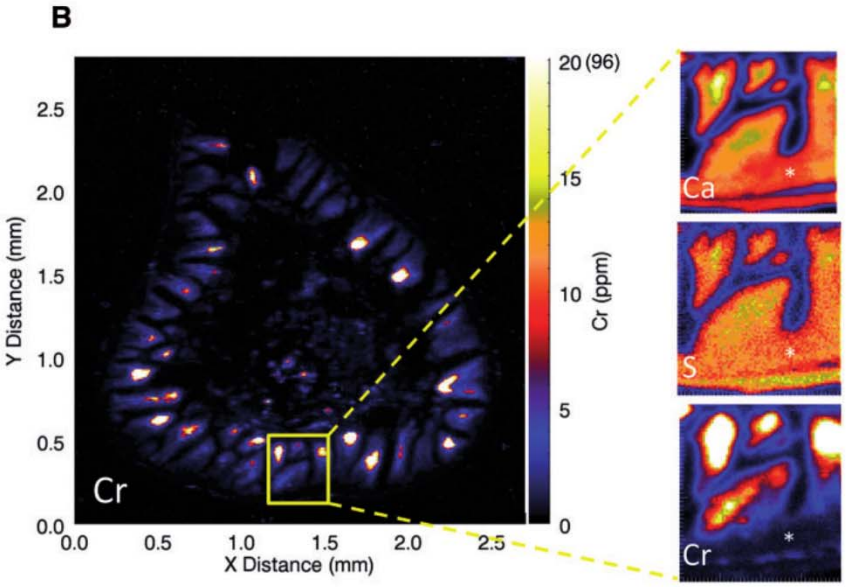
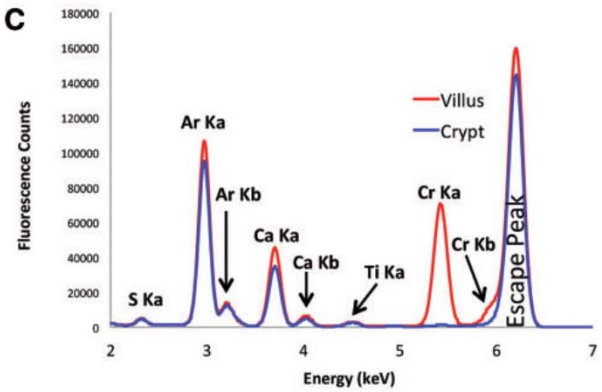
In Press: After 90 Days of Exposure to 180 ppm Cr(VI), Cr is Localized to Duodenal Villi

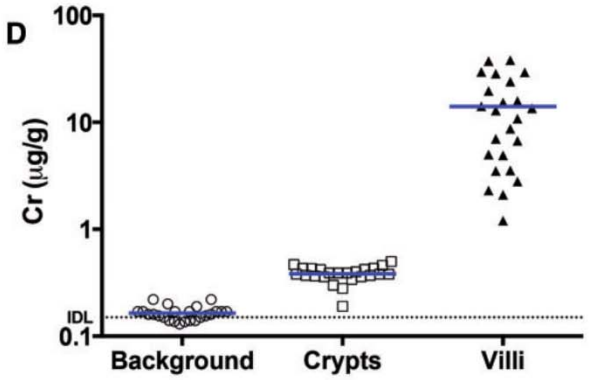


X-ray Fluorescence Mapping









Thompson et al. (Tox Sci, in press)

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In Press: After 90 Days of Exposure to 180 ppm Cr(VI), γ-H2AX is Not Elevated in Crypts

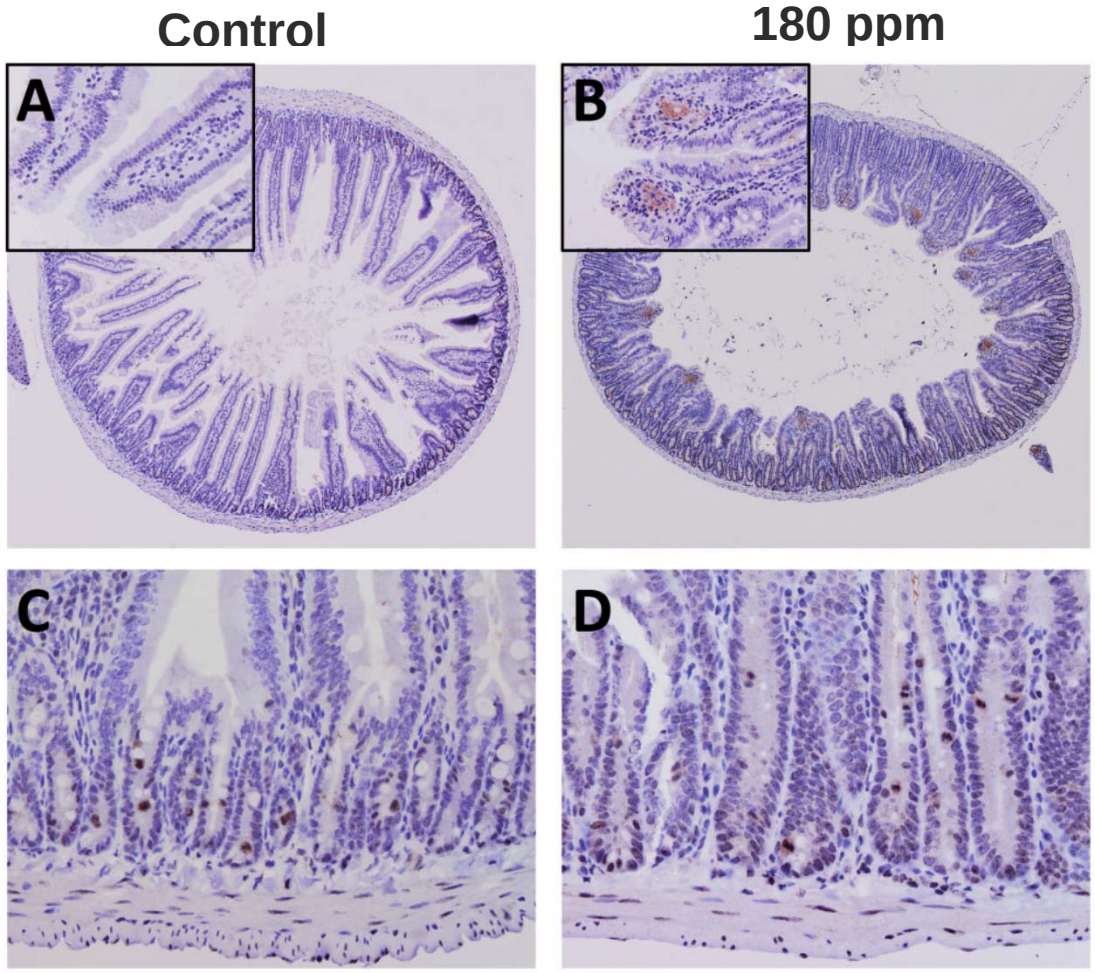
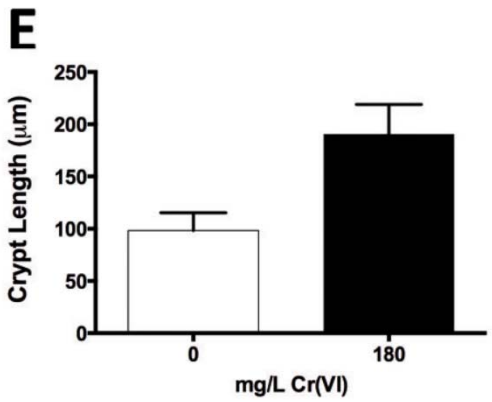


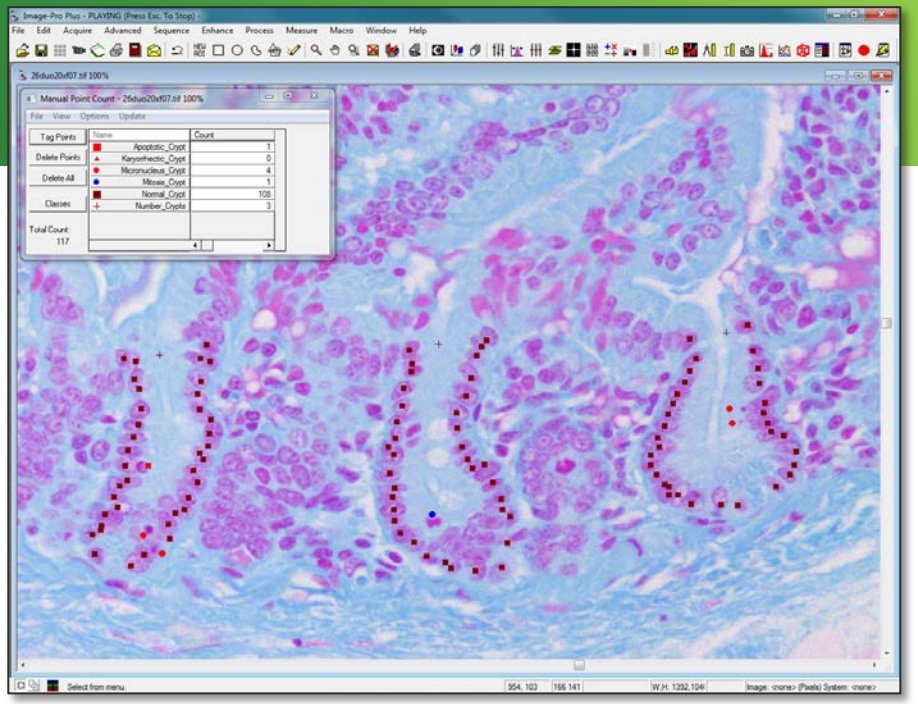
TABLE 1. Scoring of γ-H2AX Immunoreactivity

Cr(VI), mg/l	Animal no.	Crypt epithelium	Villus epithelium	Lamina propria of villus tip
0	6	2	0	0
0	7	2	0	0
0	8	2	0	0
0	9	2	0	0
0	10	2	0	0
180	482	2	1	2
180	483	2	0	2
180	484	2	1	2
180	485	2	1	2
180	486	2	2	2



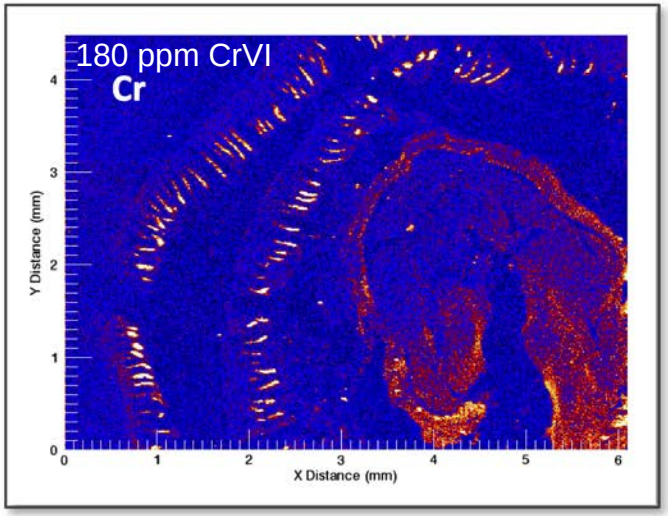
Thompson et al. (Tox Sci, in press)

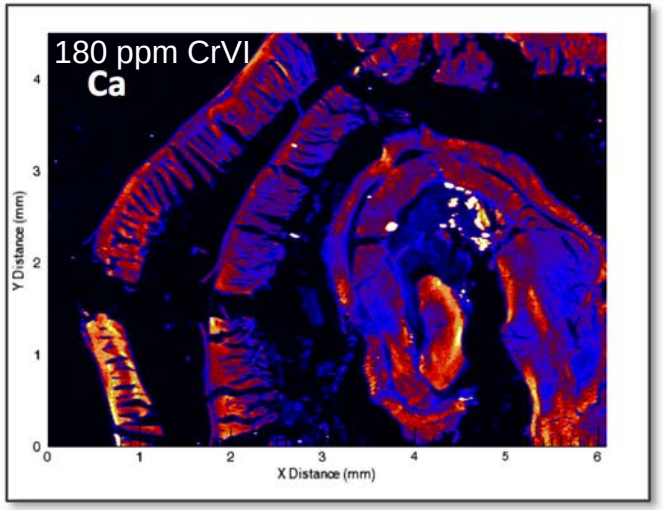
Ongoing Micronucleus Study: GLP, Swiss Roll, Pos. Contol



- **Crypt Health**
  - cell counts
  - micronuclei

- **XRF mapping**

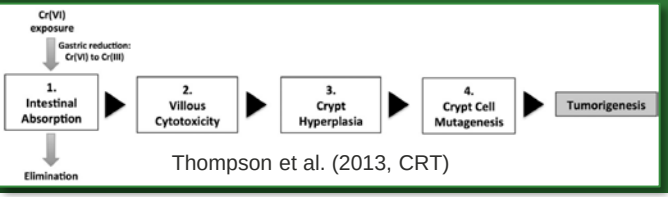




- **γ-H2AX**

in process

MOA for Intestine



Thompson et al. (2013, CRT)

Temporal

Dose-Response

Dose (ppm)	Day 8	Day 91	Two Years (NTP)
0.1	Crypt Genotox (-)	Crypt Genotox (-)/Mut (-)	Not Done
1.4	Crypt Genotox (-)	Abs (+) Crypt Genotox (-)/Mut (-)	Not Done
5	Crypt Genotox (-)	Abs (++) Crypt Genotox (-)/Mut (-)	Vill Cytotox (++) Crypt Prolif (++) Tumor (-)
20	Crypt Genotox (-)	Abs (++) Vill Cytotox (++) Crypt Prolif (+) Crypt Genotox (-)/Mut (-)	Vill Cytotox (++) Crypt Prolif (++) Tumor (+)
60	Vill Cytotox (++) Crypt Genotox (-)	Abs (++) Vill Cytotox (++) Crypt Prolif (++) Crypt Genotox (-)/Mut (-)	Vill Cytotox (++) Crypt Prolif (++) Tumor (++)
180	Abs (+) Vill Cytotox (++) Crypt Prolif (++) Crypt Genotox (-)	Abs (++) Vill Cytotox (++) Crypt Prolif (++) Crypt Genotox (-)/Mut (-)	Vill Cytotox (++) Crypt Prolif (++) Tumor (++)

\- = not seen
\+ = observed
++ = significant

Female Data

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Evidence for Cytotoxic MOA in Intestine

Modified Bradford-Hill	Supporting Evidence	Potential Inconsistent Data
Dose-response, temporal concordance	• Prolif. @ lower doses than tumors • **$BMD_{prolif} < BMD_{tumor}$** • Villous tox. before crypt prolif. • **Prolif. @ 1 wk, tumors much later** • No increase in kras @ day 91 • No increase in MN at day 8 or 91	• Some studies report genotoxicity after only 1 day of exposure
Consistency, specificity	• **Incidence of hyperplasia > tumor** • Rats: no hyperplasia & no tumors • **XRF mapping: Cr localizes to villi, not crypt** • crypts line entire intestine, but tumors observed in region of high villous absorption leading to toxicity and prolif.	• Our 90-day study found villous toxicity and crypt proliferation in rats (possibly due to higher test article intake); however this could also be considered as supporting evidence of cytotoxic MOA
Biological plausibility	• **XRF mapping: Cr mainly in villi** • **Villous enterocytes not source of intestinal tumors** • Similar MOA for intestinal carcinogens captan and folpet	• Cr(VI) can be genotoxic/mutagenic in some systems • Villous enterocytes can be coaxed into dedifferentiating in genetically engineered mice

Adapted from Meek et al. (2014) WHO/IPCS Framework on MOA/Species Concordance

Evidence for Mutagenic MOA in Intestine

Modified Bradford-Hill	Supporting Evidence	Potential Inconsistent Data
Dose-response, temporal concordance	• Some studies report in vivo genotoxicity after only 1 day of exposure	• Villus cytotox→ crypt prolif → tumors • Prolif @ 1 week • No increase in kras @ day 91 • No increase in MN @ day 8 or 91
Consistency, specificity	• Tumors in ”multiple sites, multiple species, and both sexes” • **Cr is highest in small intestine (villi)**	• Only one tumor location (SI) in mice • Only one tumor location (mouth) in rats • No early onset of tumors (not early Event) • SI tumors only in species that developed cytotox. & regenerative prolif. (i.e. mice) • **Villous enterocytes are nonprolif.** • **No aberrant foci in villi of mice, day 91** • γ-H2AX staining not elevated in crypts • Pos in vivo mutation data in transgenic rodents only at high, toxic/lethal concentrations • **Negative Big Blue Assay (oral, rats)**
Biological plausibility	• **Cr(VI) can be genotoxic/mutagenic in some systems** • Villous enterocytes can be coaxed into dedifferentiation in genetically engineered mice	• **XRF mapping: Cr not stem cell compartment** • Villous enterocytes not source of tumors • Non mutagenic MOA for other intestinal carcinogens (captan and folpet) that elicit similar phenotype

Adapted from Meek et al. (2014) WHO/IPCS Framework on MOA/Species Concordance