Science Question 4: Utility of Subchronic Histopathological Data

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ToxStrategies, Inc.

Supported by ACC

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90-Day Study in Table 3-2 Follows NTP Study Design

NTP	90-Day Study
Species/Strains: B6C3F1 mice, Fisher 344 rats	Species/Strains: B6C3F1 mice, Fisher 344 rats
Doses: 0, 14, 57, 172, 516 mg/L SDD (2-yr study doses)	Doses: 0, 0.3, 4, 14, 60, 170, 520 mg/L SDD
Research Laboratory: Southern Research Institute	Research Laboratory: Southern Research Institute

Study Design reviewed by Independent Expert Panel

Panel Member	Affiliation
Michael Dourson	TERA
Jeffery Fisher	Univ. of Georgia
David Gaylor	Gaylor & Associates
Kirk Kitchin	U.S. EPA
Bette Meek	Univ. of Ottawa
Xianglin Shi	Univ. of Kentucky
Patrick Winter	Washington Univ.

Study Objectives

- **Study was not designed to:**
  - Disprove that Cr(VI) is carcinogenic in mouse small intestine (NTP, 2008)
  - Supplant the NTP 2-yr bioassay as basis for risk assessment
  - Question the relevance of intestinal tumors to humans
- **Study was designed to:**
  - Inform the MOA for intestinal tumors in mice
    - Inclusion of lower doses and earlier time points
    - Inclusion of additional analyses (PK, pathology, biochemistry)
  - Inform which risk assessment approaches are best suited for assessing the carcinogenic risk of Cr(VI) via ingestion
  - To collect pharmacokinetic data to aid in extrapolation to humans
    - Kirman et al. (2012), rodent PBPK model
    - Kirman et al. (2013), human PBPK model
    - EPA-authored PBPK model (in peer-review)

Study Collaborators and Publications

Organization	Role
Applied Speciation	Analytical chemistry
Brooks Rand Laboratory	Analytical chemistry
Duke University Medical School	Gastric fluid samples
Environmental Standards	Analytical oversight
Experimental Pathology Laboratories	Histopathological analyses
George Washington Univ. Medical School	Kras mutation analysis
Michigan State University	Toxicogenomic analyses
National Center for Toxicological Research	Kras mutation analysis
Southern Research Institute	In-life study, histopathology, biochemical analyses
Summit Toxicology	Pharmacokinetic analysis
ToxStrategies	Study oversight, data analysis
Univ. of Cincinnati Medical School	Redox data
U.S. Army Engineer Research & Development Center	Synchrotron analyses

Publication Topic	Citation
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
Genomic Expression Profiles	2012, *Reg Tox Pharm* 64 (1)
K-*ras*/micronucleus	2013, Mut Res 754
PBPK- Rodents	2012, *CBI* 200
PBPK- Humans	2013, *CBI* 204
Iron homeostasis	2014, *Food & Chem Tox* 65
Synchrotron analysis	submitted

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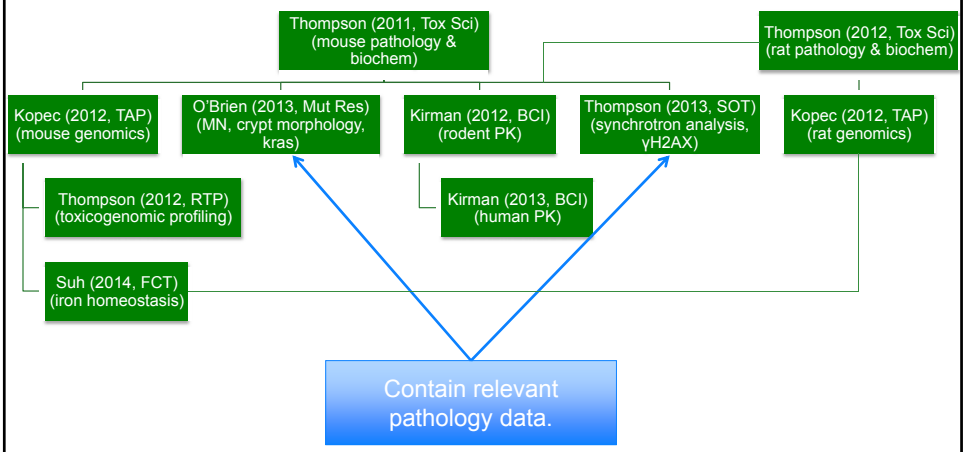
90-Day Studies Listed in Table 3-2 Provide Much More Information than Contained in the Two Cited Manuscripts



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90-Day Study in Table 3-2 (Histological Changes Occur in Villi Before Crypts)

	Incidence of Lesion						
SDD, mg/L:	0	0.3	4	14	60	170	520
Day 8							
Villous vacuolization	0/5	0/5	0/5	0/5	0/5	**3/5**	**5/5**
Crypt hyperplasia	0/5	0/5	0/5	0/5	0/5	0/5	**3/5**
Day 91							
Villous vacuolization	0/10	0/10	0/10	0/10	**5/10**	**10/10**	**7/10**
Crypt hyperplasia	0/10	0/10	0/10	0/10	0/10	**9/10**	**9/10**

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Source: Thompson et al. (2011)

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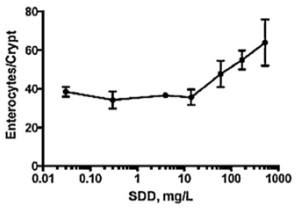
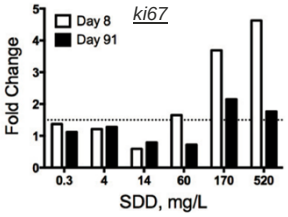
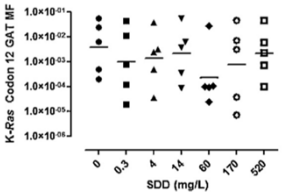
Extension of 90-Day Study in Table 3-2 (Cr6 Does not Directly Affect Crypts)

SDD, mg/L:	0	0.3	4	14	60	170	520
Crypt cells evaluated	1921	1707	1825	1420	2386	2746	3194
Crypt Micronuclei	0	0	0	0	0	0	0







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Source: O'Brien et al. (2013) and Thompson et al. (2013)

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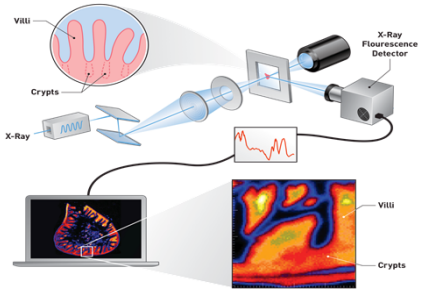
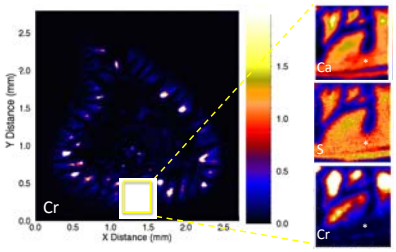
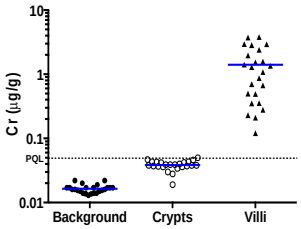
Extension of 90-Day Study in Table 3-2 (Cr Localizes to Villi and Not Crypts)

X-ray Fluorescence (Spectro)microscopy







Source: Thompson et al. (submitted)

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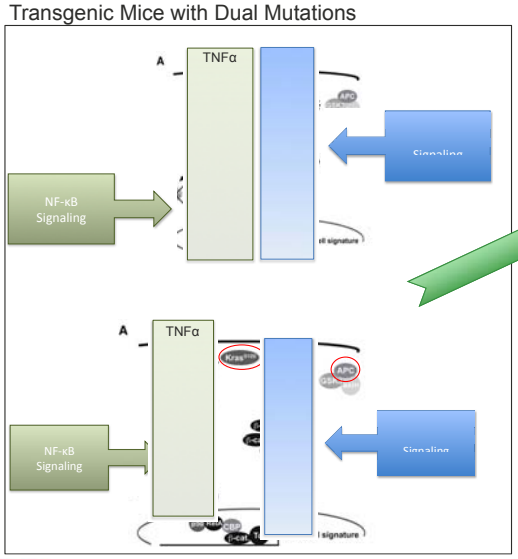
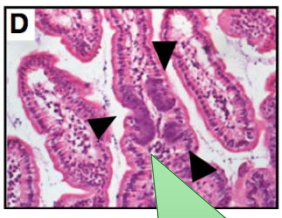
Can Tumors Arise from Villi?-Part 1

Transgenic Mice with Dual Mutations



Adapted from Schwitalla et al. (2013)

Aberrant Foci via H&E



- foci not observed in MOA study (Thompson et al., submitted)
- foci not reported in NTP studies
- TNFα protein decreased in MOA study (Thompson et al., 2011)
- TNFα mRNA decreased in MOA study (Kopec et al., 2012)

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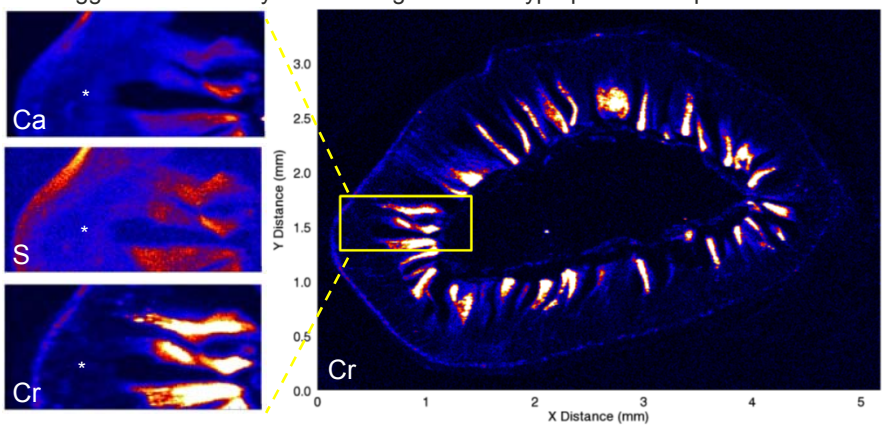
Can Tumors Arise from Villi?-Part 2

- Cr localizes to duodenal villi of rats from Thompson et al. (2012).
- Rats in NTP study likely had some Cr in villi (as evidenced by liver Cr data, for example), yet no tumors.
- Suggests that toxicity-induced regenerative hyperplasia is requisite.



Source: Thompson et al. (submitted)

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