Scientific Workshop:

Factors affecting the reduction of hexavalent chromium in the GI tract and their potential impact on evaluating the carcinogenicity of oral exposures to hexavalent chromium

Workshop Slides

9:30 am – 12:30 pm EDT
Thursday September 19 & Tuesday September 24

Sponsored by EPA's NCEA

EPA United States Environmental Protection Agency

Scientific Workshop on Factors Affecting the Reduction and Absorption of Hexavalent Chromium in the Gastrointestinal Tract

Thursday September 19 and
Wednesday September 25, 2013
9:30 am – 12:30 pm (EDT)

The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

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Chairs

Gary Ginsberg, *Connecticut Department of Public Health*
Elaina Kenyon, *US EPA (ORD/NHEERL)*

Panelists

Kim Barrett, University of California, San Diego
Max Costa, New York University
John Crison, Bristol-Myers Squibb
Silvio De Flora, University of Genoa (Italy)
Sean Hays, Summit Toxicology

Organizers

EPA/IRIS: *Catherine Gibbons, Alan Sasso, Susan Rieth, Ted Berner*
ICF: *Audrey Turley, Courtney Skuce*

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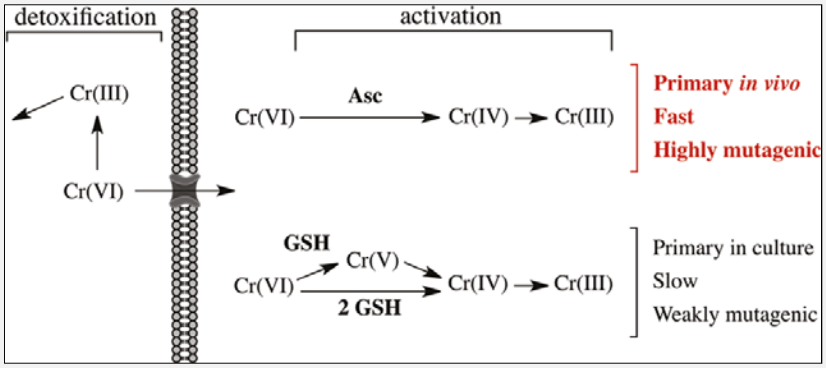
Reduction of hexavalent to trivalent chromium



Zhitkovich, *Chem Res Toxicol* 24: 1617-1629 (2011)

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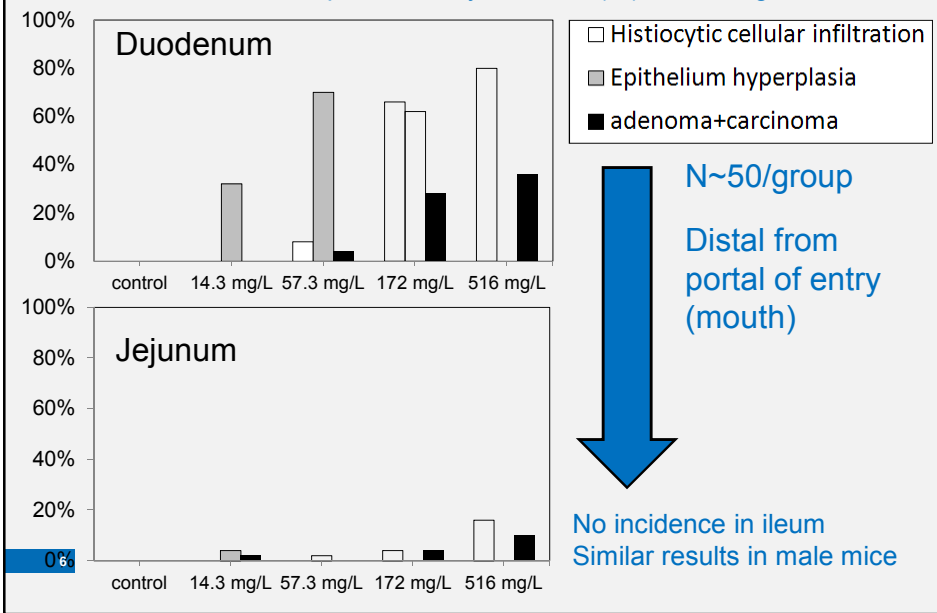
National Toxicology Program (2008)

female **MICE** exposed for 2 years to Cr(VI) in drinking water



N~50/group

Distal from portal of entry (mouth)

No incidence in ileum
Similar results in male mice

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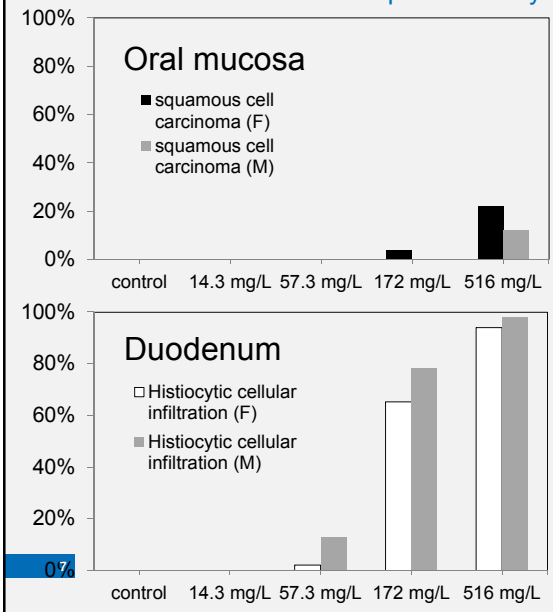
National Toxicology Program (2008)

male and female **RATS** exposed for 2 years to Cr(VI) in drinking water



N~50/group

Distal from portal of entry (mouth)

No tumors
No further distal GI effects

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Evidence in humans is limited

- Zhang & Li (1987) and reanalysis (Beaumont et al., 2008)
  - Population in China chronically exposed to drinking water heavily contaminated with Cr(VI)
  - Currently the only study in humans that indicates a somewhat elevated risk of stomach cancer
- IARC determined this single study was insufficient to constitute evidence of an association between oral exposure to Cr(VI) and stomach cancer
  - **International Agency for Research on Cancer** (2012). IARC Monographs: *A review of human carcinogens: Arsenic, metals, fibres, and dusts.*

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What do we know about physiological and biochemical processes of the GI tract?

– Can they explain similarities and differences in response between species?

– Can they identify susceptible human populations?

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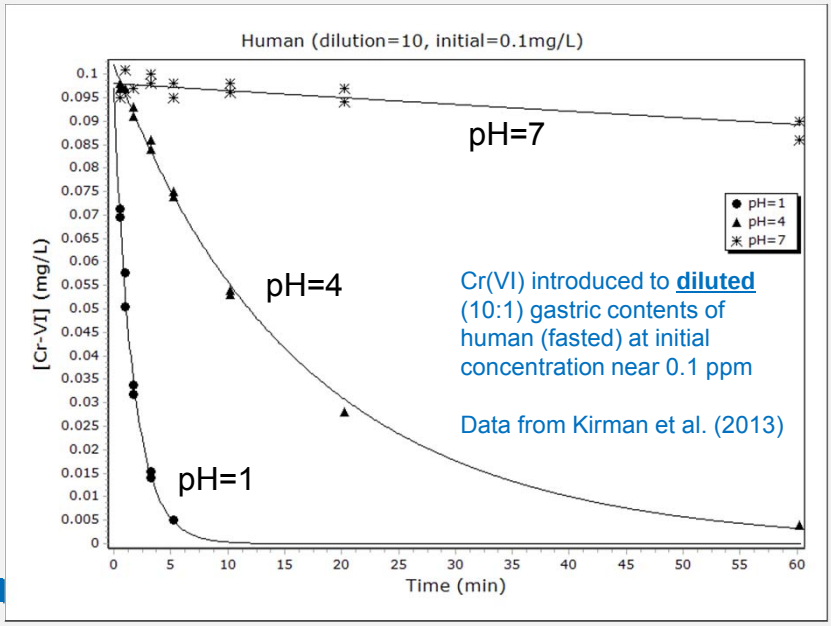
Reduction as a function of pH (*ex vivo*)



Cr(VI) introduced to **diluted** (10:1) gastric contents of human (fasted) at initial concentration near 0.1 ppm

Data from Kirman et al. (2013)

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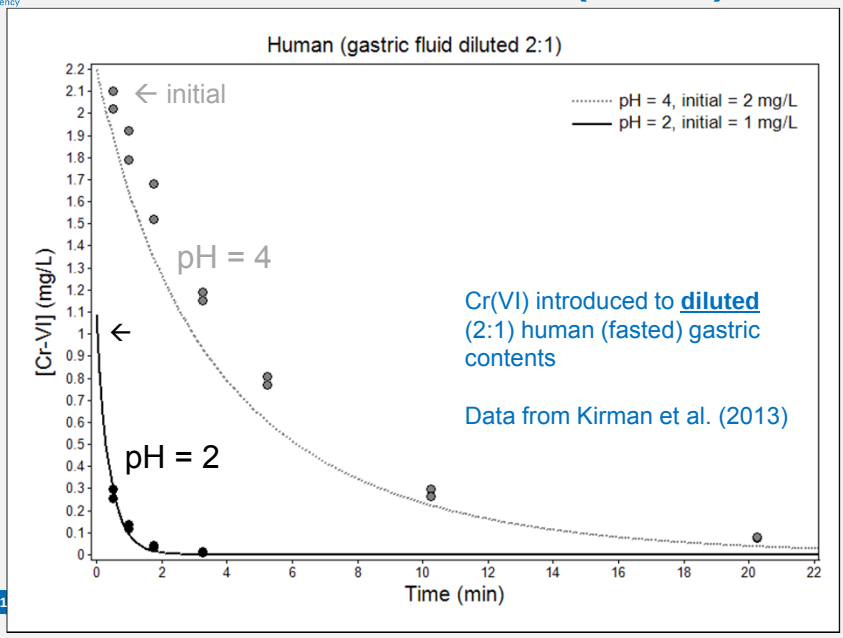
Reduction at low dilution (*ex vivo*)



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In vivo uncertainties

- Only possible to analytically measure **total** chromium in vivo
  - Total chromium = Cr(VI) + Cr(III)
- Oral ingestion of Cr(VI) leads to absorption of a Cr(VI)/Cr(III) **mixture** due to reduction
  - Difficult to know which form passed through the intestine
  - High red blood cell (RBC) to plasma ratios may indicate Cr(VI) uptake: RBCs rapidly absorb and reduce Cr(VI), "trapping" Cr(III)
- Dietary exposure to Cr(III) occurs in all species

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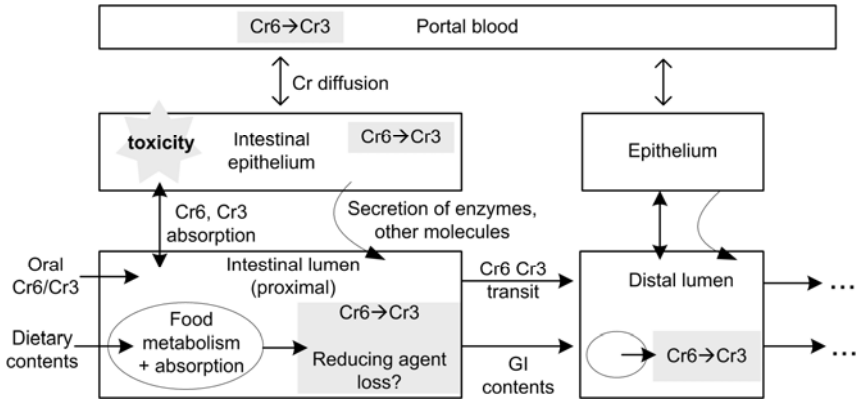
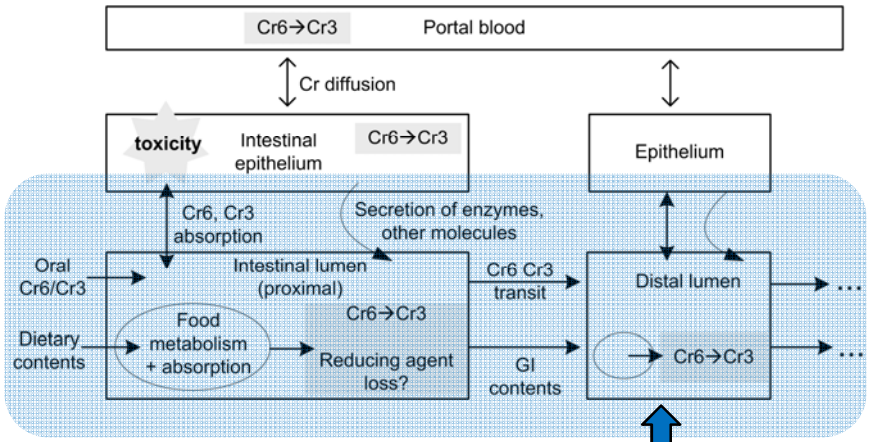
Competing transport, reduction, and uptake



- Higher **total** chromium in body following Cr(VI) exposure [compared to Cr(III)] (NTP, 2008, 2010)
- Distal chromium concentrations decrease (**duodenum** > **jejunum** > **ileum**) (Kirman et al., 2012)

Competing transport, reduction, and uptake



Workshop focus

While issues related to toxicity and susceptibility will be discussed, the primary focus will be on factors affecting Cr(VI) → Cr(III) in the GI tract and their impact on evaluating the carcinogenicity of oral exposure to Cr(VI).

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Discussion Topic 1

Regional absorption

Lead Discussant:
Dr. Max Costa

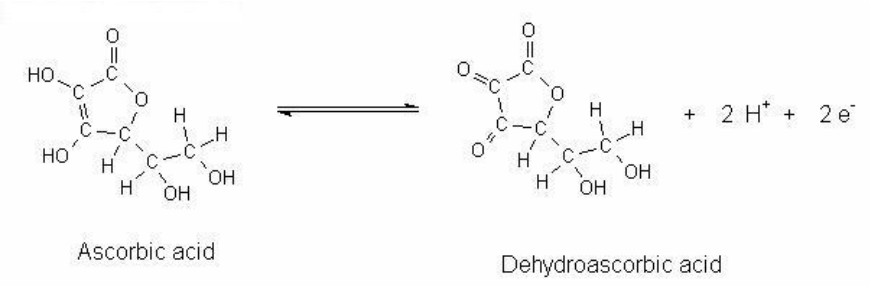


TABLE 3
Bioavailability and Tissue Chromium Measurements Following Ingestion of 5 mg Cr(VI) in Water or Reduced to Cr(III) in Orange Juice

	Peak RBC Chromium (µg/l)[a]	Peak Plasma Chromium (µg/l)[a]	Peak Urine Chromium (µg/g creat.)	Bioavailability[b] (%)
		Historical background[c]		
Mean	3.3	1.2	0.39	—
Range	1.3 (ND)–7.6	0.25 (ND)–3.9	0.07 (ND)–2.0	—
		Cr(VI) in orange juice[d]		
Mean	5.5	2.1	24	0.60
Range	5.1–6.1	1.7–2.3	18–36	0.31–0.82
		Cr(VI) in water[e]		
Mean	18	25	209	6.9
Range	14–24	5.1–57	29–585	1.2–18

[a] Peak measurements are based on first 24 h after bolus dose ingestion
[b] Bioactivity was assessed over the 2 week period following bolus dose ingestion as the cumulative amount of chromium that was excreted above historical background, divided by the total dose ingested
[c] Historical background values are the mean and range of all pre-dose measurements (n=133) from this and other studies in which the current volunteers (H4, H5, H6, H8, H9, H10) have participated. For all measurements not detected (ND), 1/2 the detection limit was used
[d] Volunteers ingested 0.5 l of 10,000 µg Cr(III) -orange juice/l within 2 min. The participants in this study were: H4, male, age 34, 92.9 kg; H8, male, age 39, 81.6 kg; H9, male, age 66, 72.5 kg and H10 (control, not included in statistics), age 35, 68kg
[e] Volunteers ingest 0.51 of 10,000 µg Cr(VI)/l within 2 min. The participants in this study were: H4, male, age 34, 92.9 kg; H5, male, age 42, 86.1 kg; H6 (control, not included in statistics), male, age 42, 65.7 kg; H8, male age 39, 81.6 kg and H10, male, age 35, 68.0 kg

TABLE 1
Tissue Levels of Cr in Male and Female Rats[a]

	Tissue[b]	Tissue concentration[c] µg/g
Chromium (VI)[d] drinking water concentration ppm of chromium		
0.45	L	0.05
	K	0.26
	B	0.67
2.2	L	0.12
	K	0.38
	B	1.4
4.5	L	0.31
	K	0.77
	B	2.3
7.7	L	0.62
	K	2.8
	B	4.2
11.2	L	1.4
	K	4.2
	B	5.0
25.0	L	5.7
	K	12.00
	B	6.4
Chromium (III)[e] drinking water concentration ppm of chromium		
25.0	L	0.38
	K	1.6
	B	0.36

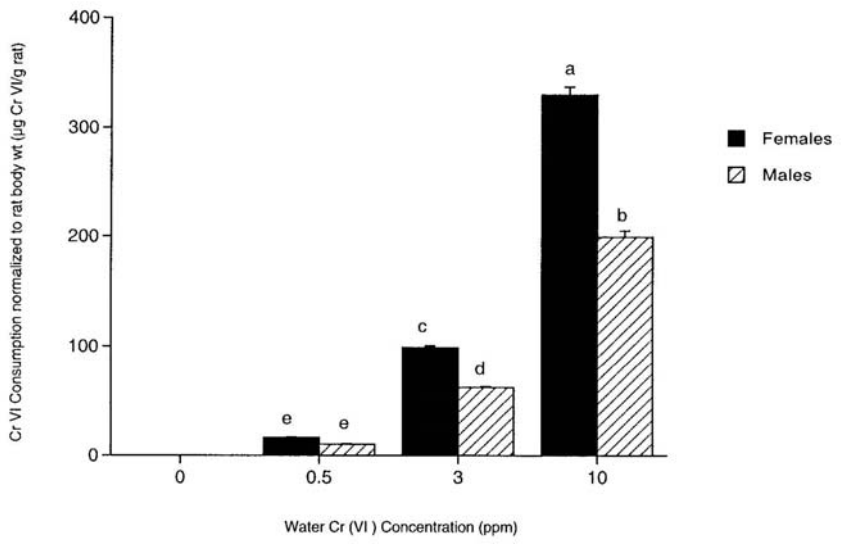
[a] Male and female rats given chromium salts in the drinking water for 1 year starting at age 34 d. $N = 8$ to 12 rats per sex and dose.
[b] L, liver; K, kidney; B, bone (femur).
[c] Mean for males and females, with background subtracted as required.
[d] As K_2CrO_4.
[e] As $CrCl_3$.

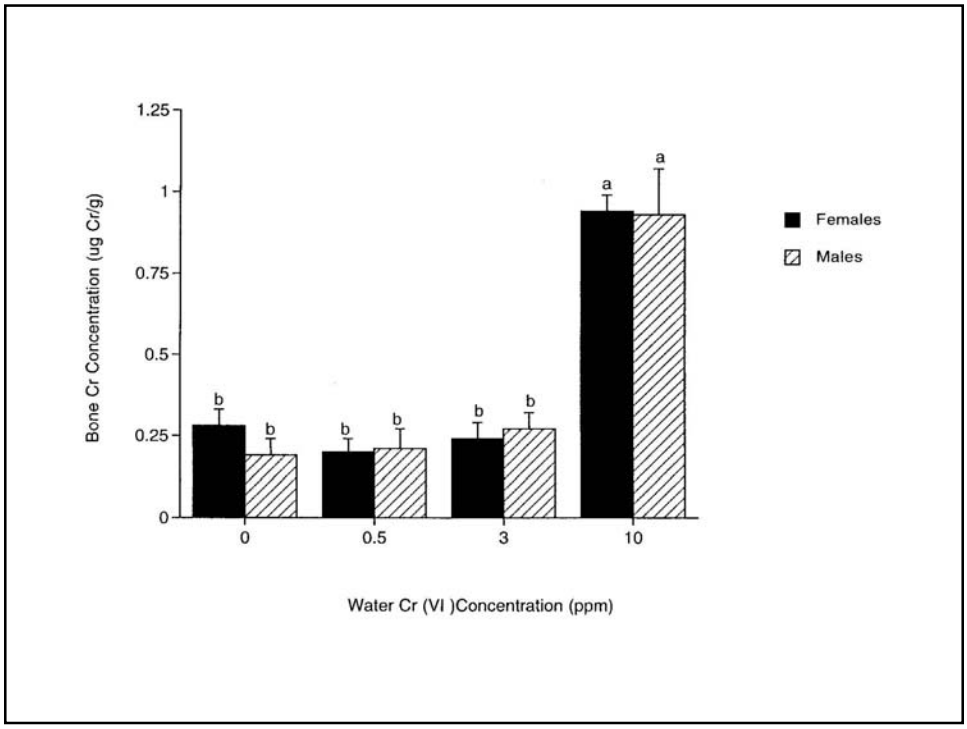
TABLE 2
Chromium Retention after Oral Exposure to Potassium Chromate in Mice and Rats

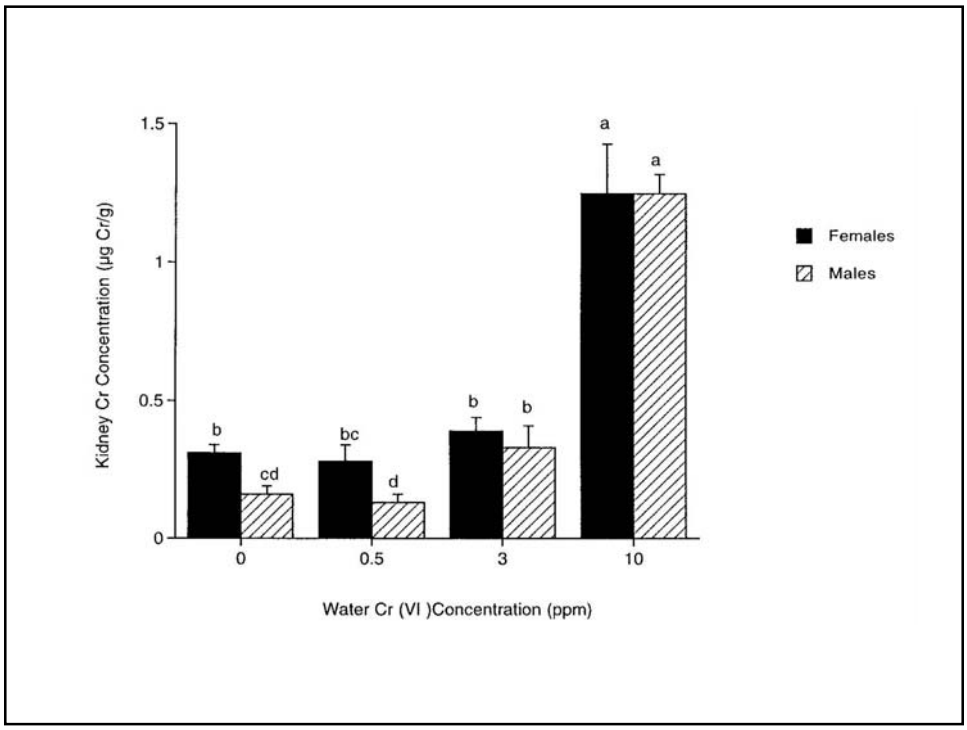
	Control		4-Wk Exposure			8-Wk Exposure		
Mice								
Liver	50[c]	0.22 ± 0.14[a]	15[c]	10.92 ± 5.48		24[c]	13.83 ± 6.06	
Femur	9	0.90 ± 0.48	4	7.43 ± 1.03		5	12.55 ± 2.99	
Spleen	14	0.53 ± 0.38	4	5.04 ± 1.45		5	10.09 ± 2.50	
Kidney	16	0.24 ± 0.14	15	3.77 ± 0.99		13	4.72 ± 0.68	
Lung	14	0.24 ± 0.12	4	0.99 ± 0.10		4	1.08 ± 0.26	
Heart	10	0.32 ± 0.15	4	0.80 ± 0.23		5	1.02 ± 0.20	
Muscle	13	0.32 ± 0.23	4	1.12 ± 0.37		5	0.60 ± 0.25	
Blood	7	0.14 ± 0.05	4	0.71 ± 0.07		1	0.42 ± 0.04	
Rats								
Kidney	11	0.34 ± 0.20	7	8.62 ± 2.40	0.4	8	9.49 ± 4.38	0.5
Spleen	11	0.43 ± 0.20	7	3.65 ± 1.87	1.4	8	4.38 ± 0.84	2.3
Liver	10[c]	0.19 ± 0.14	7[c]	3.32 ± 0.93	3.3[b]	8[c]	3.59 ± 0.73	3.9[b]
Femur	15	1.00 ± 0.46	7	1.85 ± 0.46	4.0	7	1.78 ± 0.99	7.1
Lung	11	0.39 ± 0.43	6	1.10 ± 0.38	0.9	7	0.67 ± 0.24	1.6
Heart	12	0.38 ± 0.22	5	0.52 ± 0.12	1.5	8	1.05 ± 0.19	1.0
Blood	9	0.19 ± 0.17	7	0.73 ± 0.15	1.0	7	0.58 ± 0.13	0.7
Muscle	13	0.24 ± 0.14	4	0.19 ± 0.10	5.9	7	0.17 ± 1.10	3.5

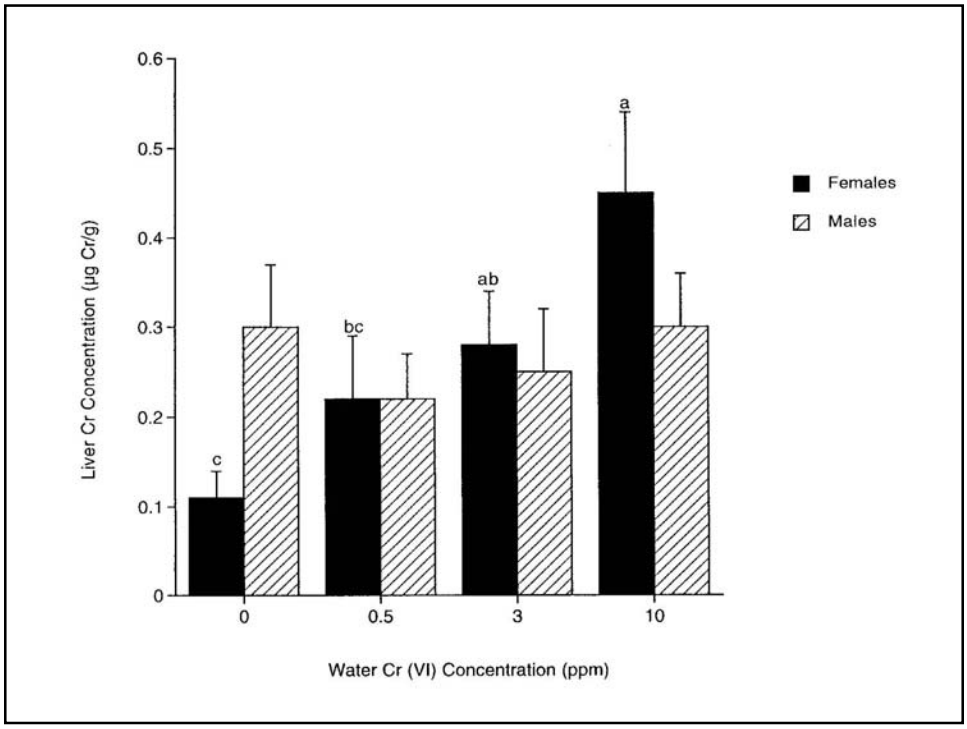
[a] Arithmetic mean ± SD.
[b] Ratio mice/rats.
[c] Number of samples taken from separate animals.

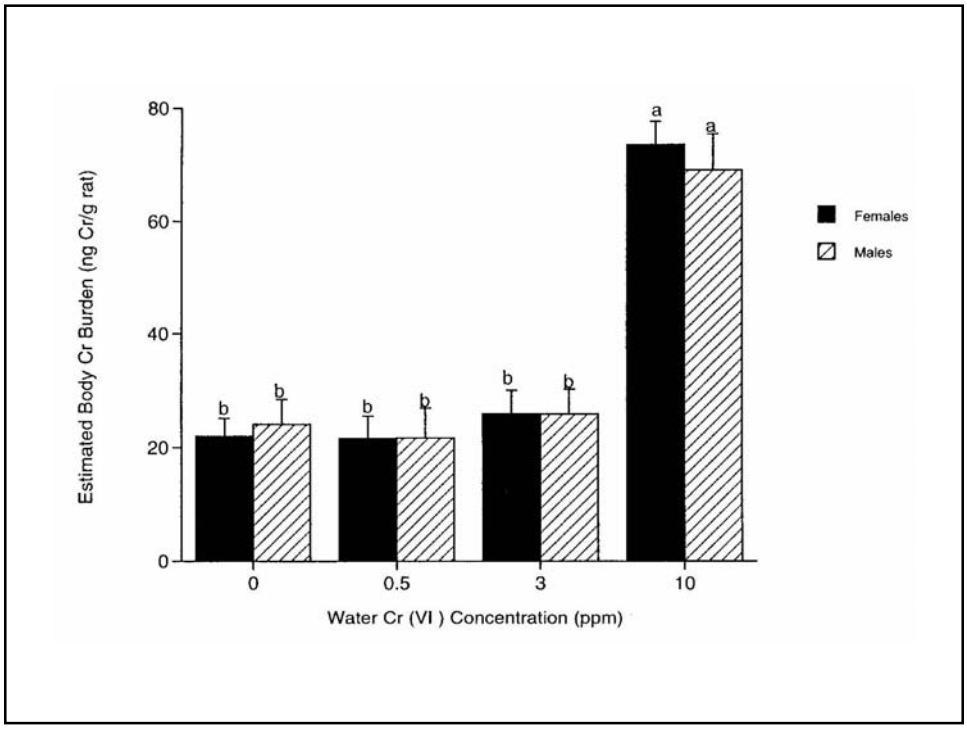
Note: Values shown in the table represent µg Cr/g wet tissue weight, and for blood µg Cr/ml. Dose of Cr(VI) was 8 mg/kg body weight (data from Reference 10). K_2CrO_4 was given to animals in their drinking water.




Bone Cr Concentration (ug Cr/g)
1.25
1
0.75
0.5
0.25
0
b
b
b
b
b
b
a
a
0
0.5
3
10
Water Cr (VI)Concentration (ppm)
Females
Males


Kidney Cr Concentration (µg Cr/g)
1.5
1
0.5
0
b
cd
bc
d
b
b
a
a
0
0.5
3
10
Water Cr (VI)Concentration (ppm)
Females
Males


Liver Cr Concentration (µg Cr/g)
0.6
0.5
0.4
0.3
0.2
0.1
0
c
bc
ab
a
0
0.5
3
10
Water Cr (VI) Concentration (ppm)
Females
Males


Estimated Body Cr Burden (ng Cr/g rat)
80
60
40
20
0
b
b
b
b
b
b
a
a
0
0.5
3
10
Water Cr (VI) Concentration (ppm)
Females
Males

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Discussion Topic 1

Regional absorption

Discussion

Topic 1

Sean Hays

90-day Studies: SDD Dose

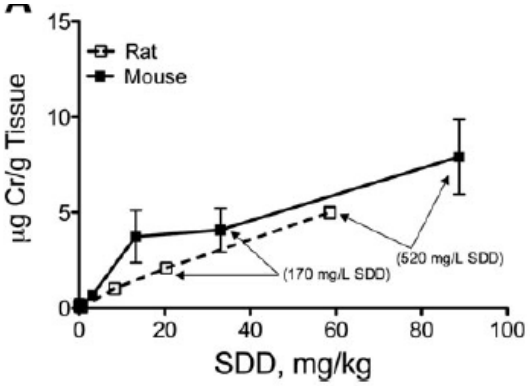
TABLE 2
Average Daily Dose of Ingested SDD

	SDD (mg/kg)					
SDD, mg/l (nominal)	0.3	4	14	60	170	520
Rat						
Average daily dose (day 8)	0.06	0.8	ND	12.1	29.9	80.9
Average daily dose (day 91)	0.05	0.6	ND	8.3	20.4	58.6
Mouse[a]						
Average daily dose (day 8)	0.08	1.1	3.3	14.0	37.4	86.8
Average daily dose (day 91)	0.07	0.9	3.1	13.2	33.0	88.7

Note. ND, not done.
[a]Data for mouse body weight, intake, and dose are taken from Thompson *et al*. (2011b).

Source: Thompson et al. 2012

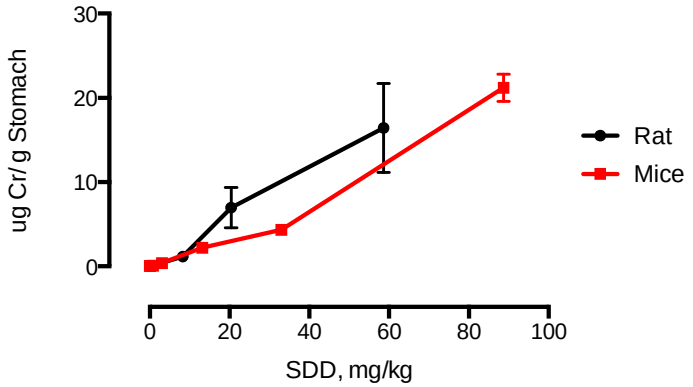
Total Cr in Oral Mucosa



- Cr levels are slightly higher in mice compared to rats
- Significantly elevated from controls at ≥ 60 mg/l SDD for mice and rats

Source: Thompson et al. 2012

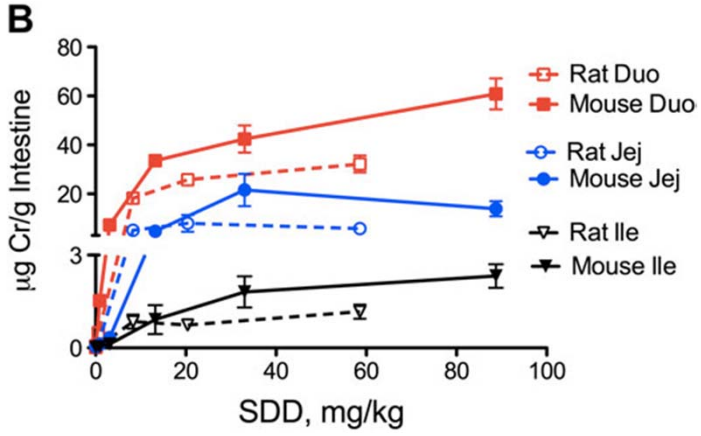
Total Cr in Glandular Stomach



Significant from controls at ≥ 170 mg/l SDD

Source: Thompson et al. 2012

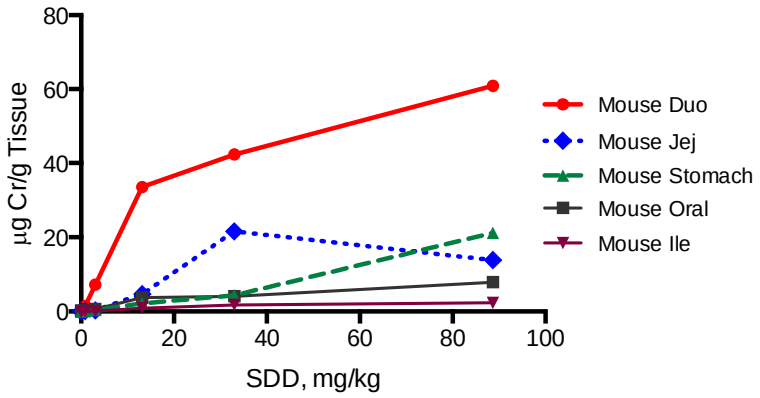
Total Cr in Intestine

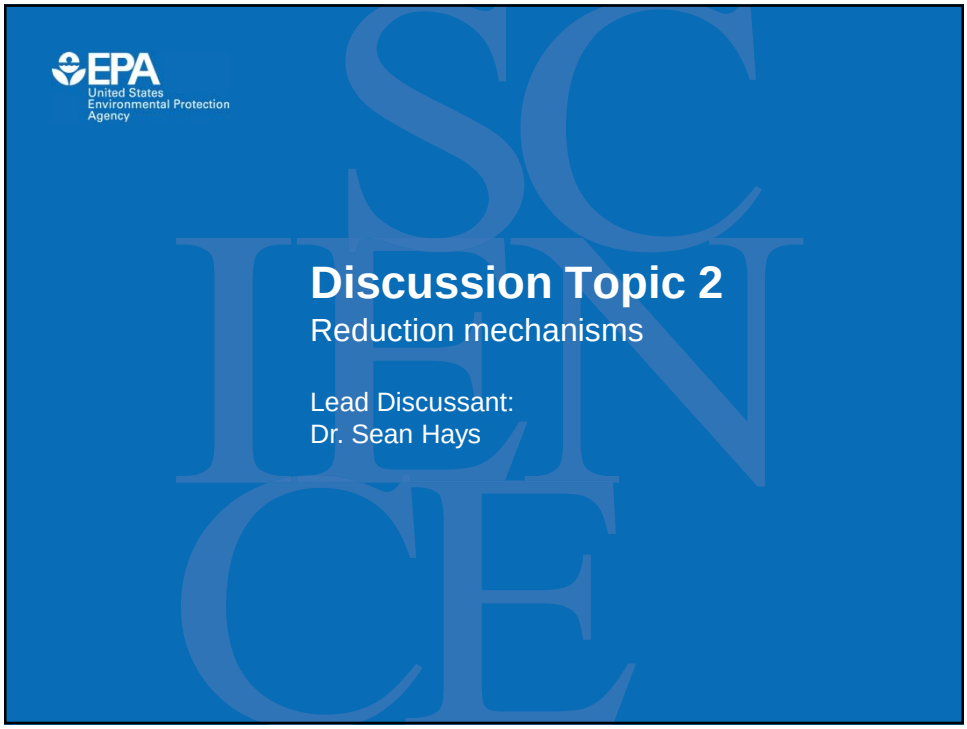


- Cr levels are slightly higher in the more proximal portions of the intestine
- Statistically significant from control animals at ≥ 60 mg/l SDD for duodenum and jejunum
- For ileum, significant increases occur at ≥ 520 mg/l SDD

Source: Thompson et al. 2012

Tissue Cr Across the GI Tract in Mice





Topic 2: Reduction of CrVI in GI Fluids

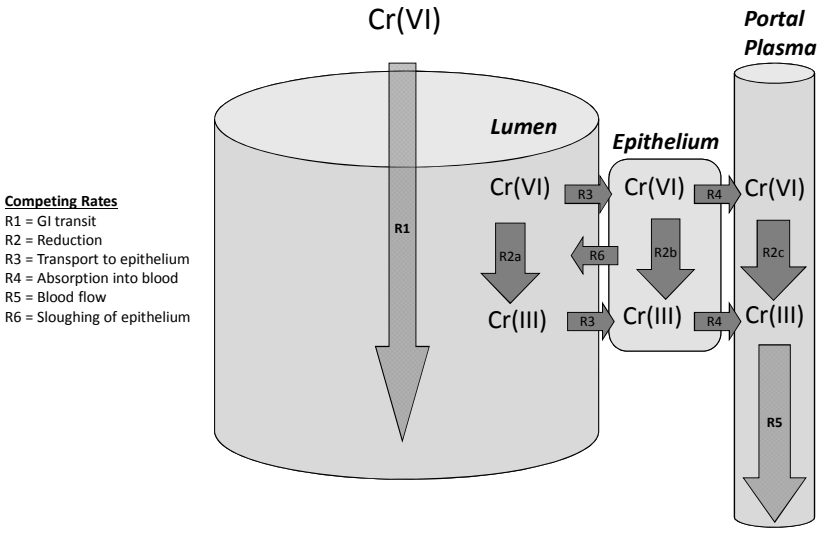
Sean M. Hays, PhD

Summit Toxicology
University of Colorado, School of Public Health
Colorado State University, Department of Chemical Engineering

Charge Questions

- Is it possible to **significantly deplete or overwhelm** any of these **reducing agents** in **small rodents** by administering repeated doses of a xenobiotic that undergoes a reduction reaction? Under the conditions of the NTP bioassay, YES.
- Is it possible to **deplete or saturate reduction capacity in humans**? Probably not at relevant drinking water concentrations.
- It's a matter of 'dose' and rates of reduction, transit and absorption….which all factor into assessing extent of CrVI delivery to the small intestine.

Conceptual Model For Cr in the GI Tract



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Modeling of CrVI Reduction

- Most have modeled using mixed second-order kinetics
- RE + CrVI → CrIII + RE_O

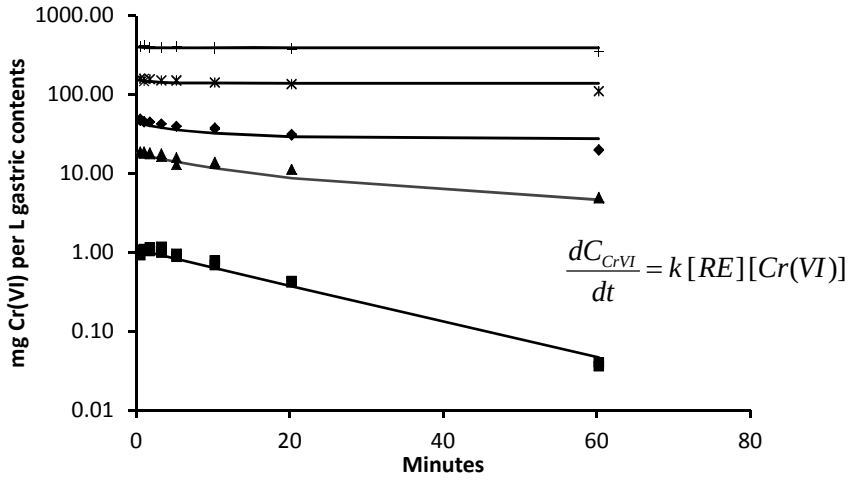
$$\frac{dC_{CrVI}}{dt} = k\,[RE][Cr(VI)]$$

  - As CrVI and/or RE are depleted, reaction slows
  - If CrVI << RE
    - CrVI is fully consumed
    - Rxn is first-order with respect to CrVI
  - If CrVI >> RE
    - CrVI is left after reducing agents are consumed
    - Rxn is not first-order with respect to CrVI

Modeling CrVI Reduction:
(Connett, 1983)

Reductant	Second order rate constant ($M^{-1}min^{-1}$)
Cysteine	75.3 ± 6.2
Cysteamine	62.9 ± 3
Ascorbate	36.1 ± 1.2
Glutathione (first phase)	>26
Unithiol	26 ± 1.6
Penicillamine	20.9 ± 4.1
Dithiothreitol	17.3 ± 1.8
Mercaptoethanol	5.4 ± 0.2
Lipoic Acid	4.8 ± 0.5
Glutathione (final phase)	4.1 ± 1.5
2,3-Dimercaptosuccinic Acid	3.8 ± 0.2
Thiolactic Acid	3.1 ± 0.2

Mouse Stomach Fluid



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Model for Cr(VI) Reduction in Rodent Stomach Fluid

$$\frac{dC_{CrVI}}{dt} = k[RE][Cr(VI)]$$

- RE – Reductants; 16 mg/L stomach contents (mice and rats)
- k=0.2 and 0.3 L mg^{-1} hr^{-1} in mice and rats, respectively

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Cr(VI) Reduction based on Cr(VI) Loading in Gastric Fluid

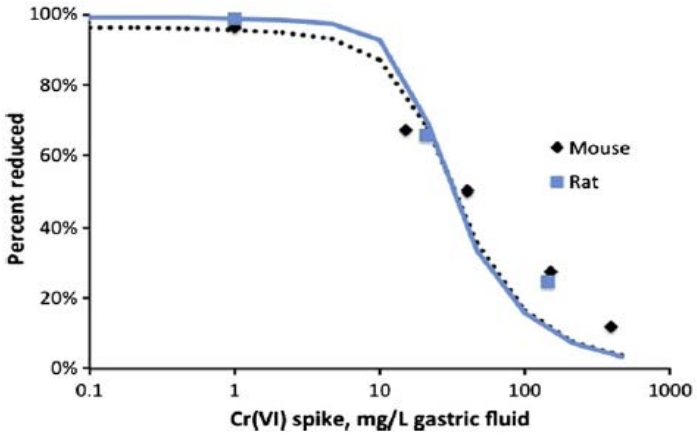
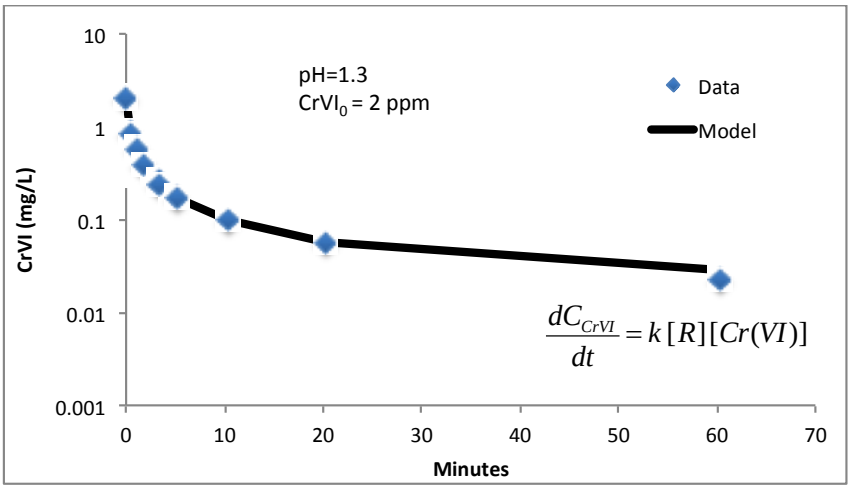
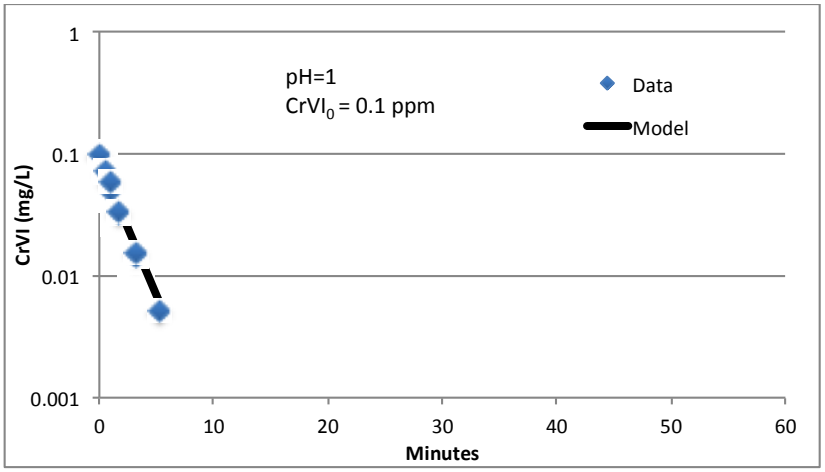


Fig. 2. Percent of Cr(VI) reduced within 60 min vs. initial loading of Cr(VI) in stomach contents of mice and rats (mg Cr(VI) L^{-1} stomach contents). Two replicate measurements using SIDMS were taken at 60 min after initial loading. Results presented herein are averages of each replicate.

Human



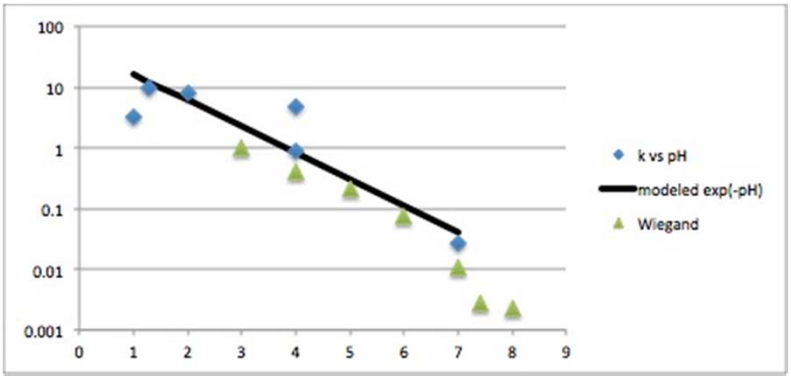
Human



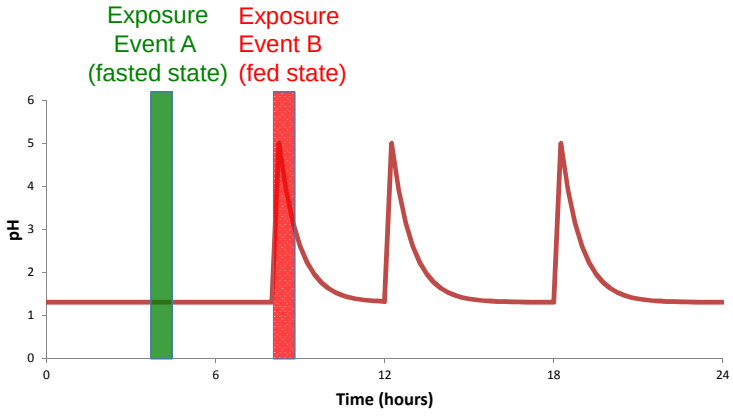
Reduction Capacity of CrVI

- Rodents ~ 16 mg/L stomach contents (Proctor et al., 2012)
- Humans
  - Fasted: ~ 4 – 10 mg/L (Kirman et al., 2013)
  - Fed: ~ 10 – 60 mg/L (DeFlora et al., 1987)

pH Dependence: Human Stomach Fluid



Exposure Timing



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

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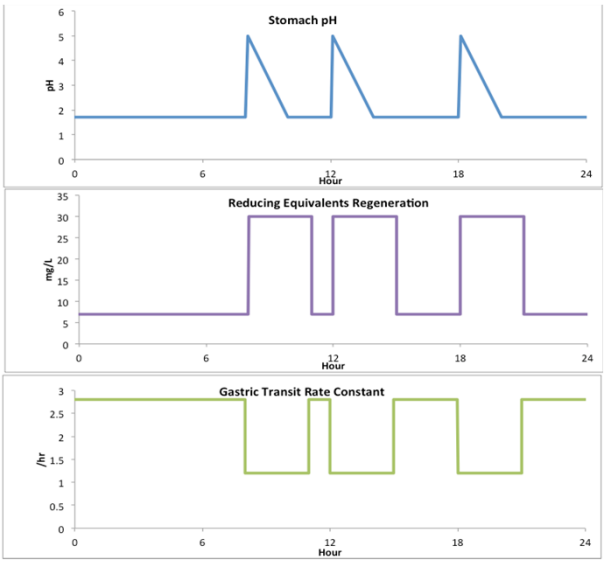
Modeling Various Competing Factors



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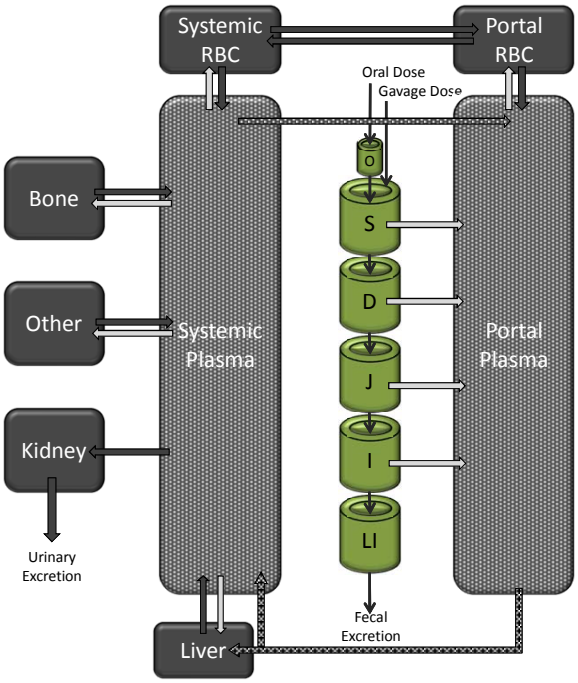
Kirman et al. (2013)

PBPK Model Structure



Kirman et al. (2013)

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Charge Questions

- Is it possible to **significantly deplete or overwhelm** any of these **reducing agents** in **small rodents** by administering repeated doses of a xenobiotic that undergoes a reduction reaction? Under the conditions of the NTP bioassay, YES.
- Is it possible to **deplete or saturate reduction capacity in humans**? Probably not at relevant drinking water concentrations.
- It's a matter of 'dose' and rates of reduction, transit and absorption….which all factor into assessing extent of CrVI delivery to the small intestine.

Charge Questions

- Are there examples in the literature or based on your experience (aside from hexavalent chromium) where the saturation or depletion of enzymatic or other non-enzymatic molecules (not necessarily limited to reducing agents) in the gastrointestinal tract lumen occurred following ingestion of pharmaceuticals, essential elements, or toxic chemicals?
- Which reducing agents in the gastrointestinal tract lumen are most at risk of being inhibited, saturated, or depleted by xenobiotics?

EXTRAS

pH Dependence:

(A) GSH (Weigand 1984), (B) Ascorbate (Xu et al. 2004)

(A)

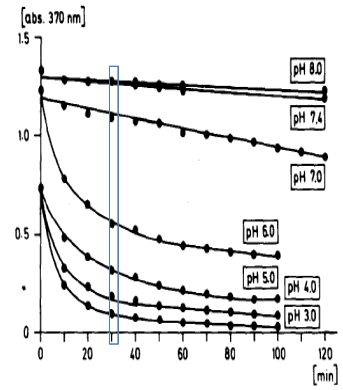


Fig. 2. Reduction of Cr(VI) by GSH at "stoichiometric conditions" (1:3) and different pH values.

(B)

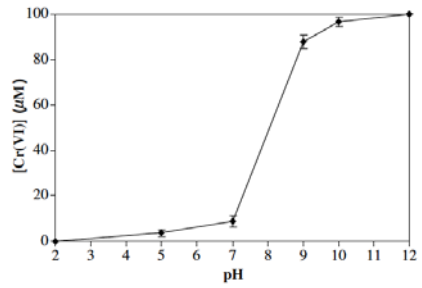
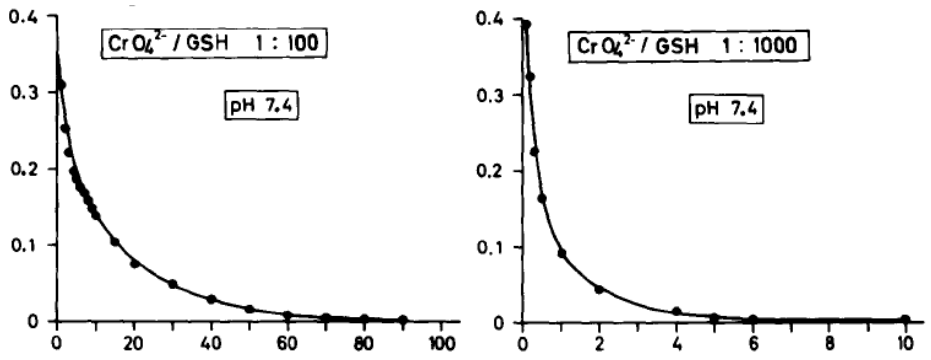


Fig. 3. Effect of pH on the reduction of Cr(VI). ([Cr(VI)] = 100 μM, [Vc] = 300 μM, temperature = 25 °C, reaction time = 30 min.) The error bars represent standard deviation of the mean.

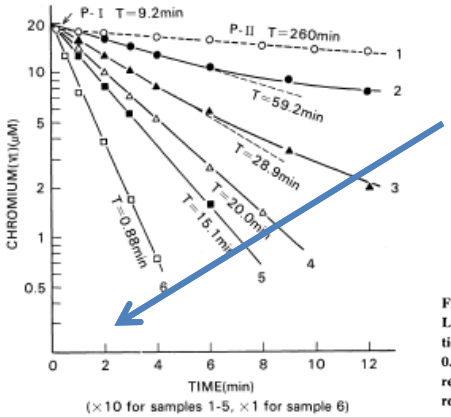
CrVI:GSH Ratio Dependence

Weigand 1984: (note difference in x-axis scale)



CrVI:Ascorbate Ratio Dependence

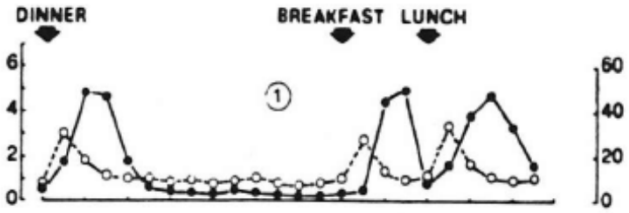
(Suzuki 1990)



Decreasing CrVI:ascorbate ratio

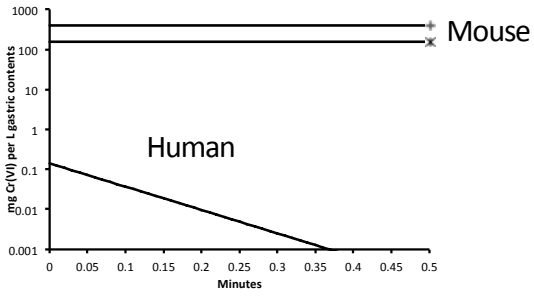
Fig. 2. Semi-logarithmic plots of residual chromium(VI) in GSH and L-AsA solution (pH 7.4) against incubation time. The initial concentrations of the reductants in samples 1, 2, 3, 4, 5 and 6 were 2 mM GSH, 0.02, 0.05, 0.06, 0.1 and 2 mM L-AsA, respectively. P-I, P-II and T represent the first and second phases and half-life of chromium(VI), respectively. Each point represents mean of two or three measurements.

DeFlora et al. 1987

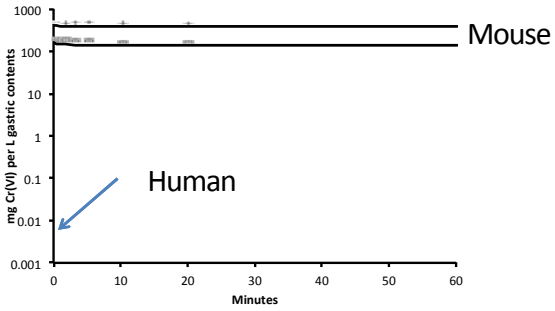


- Measured amount reduced after 60 min of incubation.
- Not measures of rates of reduction. Rather, measures of total mass of CrVI reduced by stomach fluid, despite being reported by DeFlora as ug/ml/h.
- DeFlora et al. 1987: “the reaction was rather rapid, the top levels of reduction being attained after 10-20 min”.

Reduction Rates of Cr(VI) in Stomach Fluid: Mouse v Human



Reduction Rates of Cr(VI) in Stomach Fluid: Mouse v Human



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Discussion Topic 3

Gastrointestinal pharmacokinetics

Lead Discussant:
Dr. John Crison

Physiologically Based Pharmacokinetic Modeling

Sept. 19, 2013

Bristol-Myers Squibb 60

Physiologically Based Pharmacokinetic Modeling

PBPK Models:

- **Provide a mechanistic understanding of the absorption, distribution, metabolism, and excretion.**
- **Commercial software is available.**
- **Specialized PBPK models may be necessary for specific problems not addressed with commercial software.**

Examples of Commercial PBPK Software

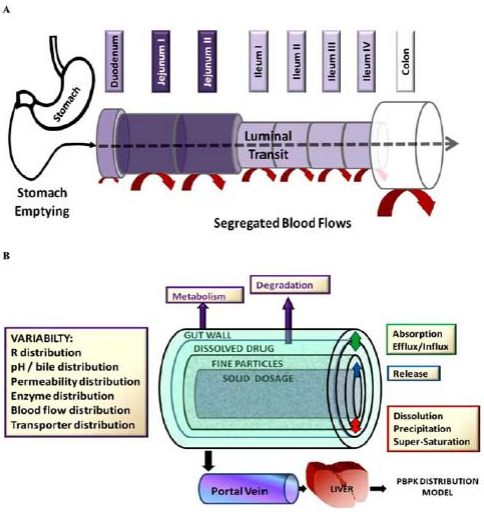
GastroPlus™ (Simulations Plus) and Simcyp™ (Certara)

- **Well defined GI tract that includes transit, pH and permeability changes, metabolism, and active transport, etc.**
- **Includes mathematical descriptions of other relevant tissues.**

Predictive Capabilities

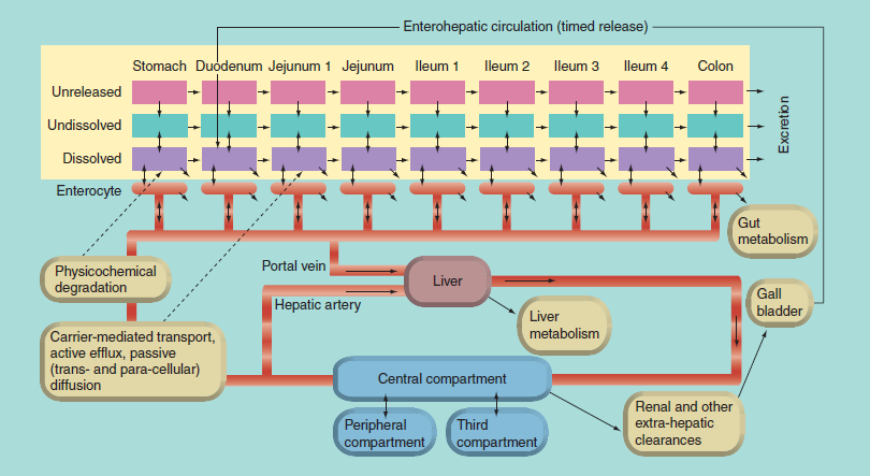
PBPK models are mechanistic and can be predictive providing there is adequate input data to describe the absorption, distribution and clearance.

Simcyp™ ADAM Model (Advanced Dissolution, Absorption, and Metabolism)



Darwich, A.S., et al, Current Drug Metabolism, 2010, 11:716-729.

GastroPlus™ ACAT Model (Advanced Compartmental Absorption Transit)



Brown, J., et al, Therapeutic Delivery, 2012, 3(9):1047-1059.

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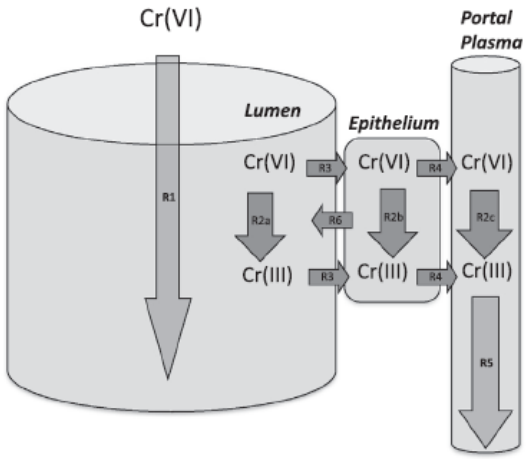
Non-Commercial Models



R1 = GI Transit
R2 = Cr6 reduction
R3 = Cr transport
R4 = Cr absorption (blood)
R5 = Cr transit (portal)
R6 = sloughed cells

Kirman, C.R., et al, Chemico-Biological Interactions 2013, 204:13-27.

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Review and Analysis of Gastric Reduction

by Dr. Silvio De Flora

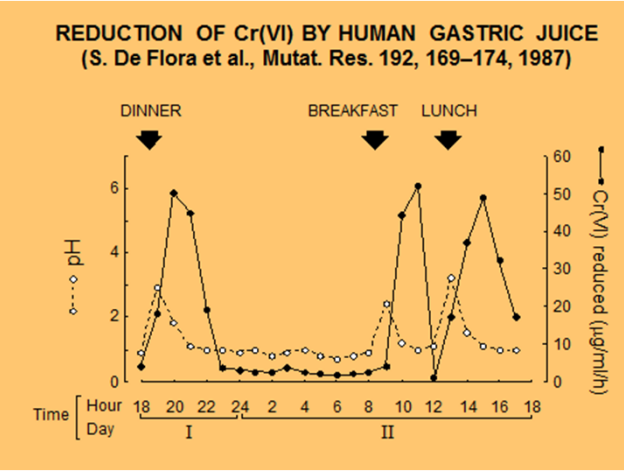
Presented by:
Dr. Sean Hays

Brief history of Cr(VI) studies in the GI tract

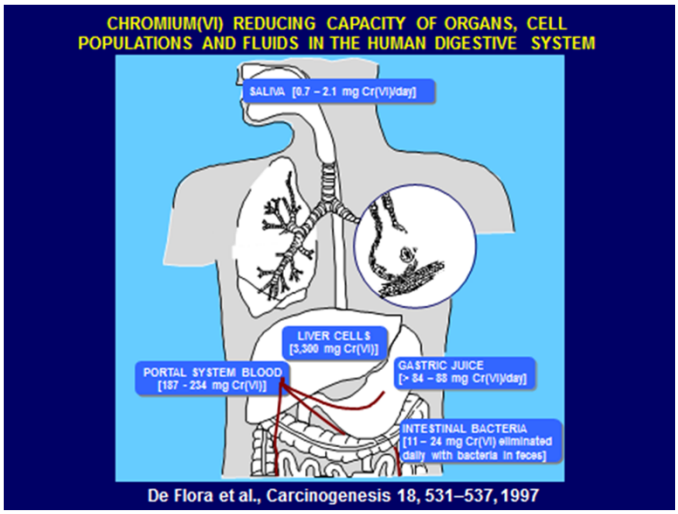
- Donaldson and Barreras (1966)- First observed Cr(VI) reduction in human gastric juice
- De Flora and Boido (1980)- Mutagenicity of some compounds may be impacted by pre-incubation with human gastric juice
  - For Cr(VI), gastric juice sharply decreased mutagenicity
  - Mutagenicity attenuated by raising pH
- De Flora et al. (1987)- nasogastric tube study
  - Human gastric juice extracted over 24 hrs
  - Measured pH, Cr(VI) reduction capacity, mutagenicity

Circadian reduction of Cr(VI) in human gastric environment [De Flora et al. (1987)]

- Nasogastric tube positioned for 24h in stomachs of 17 subjects; hourly samples of gastric juice
  - Dietary conditions standardized for all subjects
  - Total of 428 gastric juice samples analyzed for Cr(VI) reduction and mutagenicity; pH also monitored
  - s-diphenylcarbazide (DPC) colorimetric method for reduction; highly sensitive *Salmonella typhimurium* strain TA102 for Ames mutagenicity assay
- All of the 428 gastric samples were capable of reducing Cr(VI)



- Baseline Cr(VI) reduction: 8.3 +/- 4.7 µg Cr(VI)/mL
- Fed-state reduction : 31.4 +/- 6.7 (~50-60 µg Cr(VI)/mL max)
- Cr(VI) reduction complete within 10-20 min; most or reaction <1 minute
- Gastric juice secretions promoted reduction, inhibited mutagenicity



Estimation of Cr(VI) reduced daily by gastric juice (and other organs)

- 1000-1500 mL gastric juice secreted daily (fasting), plus 800 mL after each meal
- [8.3 µg/mL] x [1000-1500 mL] + [31 µg/mL x 800 mL] x 3 meals
- Overall Cr(VI) reduction by gastric juice: 84-88 mg per day

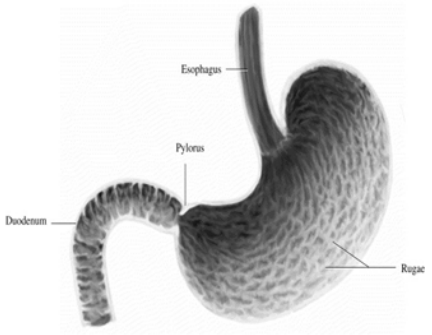
Other notes

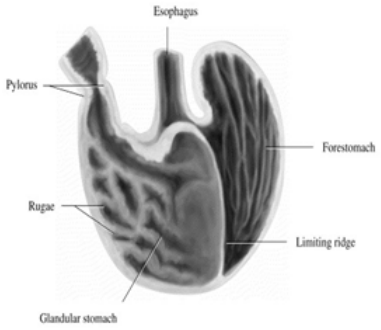
- These studies likely **underestimate** the actual Cr(VI) reducing ability in total gastric contents
  - Gastric samples centrifuged to remove food residues
  - Food/beverage, and intestinal bacteria/GSH reduce Cr(VI)
- 24-hour continuous sampling of human gastric contents no longer permitted
  - Ongoing studies of donated gastric samples confirm prior results (10 and 20 µg Cr(VI)/mL for fasted and post-meal)
- Other components besides acid reduce Cr(VI)
  - Cr(VI) reduction was enhanced by administering gastric secretion stimulators, decreased by anti-secretory drugs

Reduction and absorption of Cr(VI) in humans

- **On Day 1**, a study suggested that Cr(VI) can be absorbed after oral ingestion by humans, based on distribution of total chromium in RBC, plasma, and urine (J.R. Kuykendall et al., 1996)
  - 4 adult volunteers (fasted) drank a bolus 500 mL water containing 5 mg Cr(VI) in 2 minutes
- Four independent *ex vivo* studies indicated that the fasted human gastric juice reduces ~10 µg Cr(VI)/mL. Since the fasted human stomach contains ~25 mL gastric juice, they will reduce 250 µg (0.25 mg) Cr(VI).
  - Only a small part of the ingested 5mg Cr(VI) could be reduced by stomach
- The half-time for water emptying by the stomach is ~10 minutes, but the rate of emptying is proportional to amount in the stomach. It is likely that adding 500 mL water to 25 mL gastric juice in 2 minutes resulted in rapid emptying
- In conclusion, the reducing capacity of the human gastric juice is extremely high (>80 mg/day) but is not infinite. Under extreme conditions, which are quite unrealistic, the reduction capacity of gastric juice can be exceeded

Species differences





Humans

- pH = 1-2
- Single secretory region with numerous folds (rugae)
- Slower transit and emptying time
- Small intestine: mostly jejunum
  - Small intestine tumors very rare

Rodents

- pH = 3-5
- Two distinctive regions: forestomach devoid of glands and only stores food (60% of SA)
- No post-meal peaks of gastric juice
- Longer relative length of duodenum

Figure from DeSesso and Jacobson (2001)

NTP (2008) study

- No stomach tumors observed in rodents despite potential susceptibilities of stomach tissue
- Statistically significant increase only at highest concentration in males, two highest in females
  - No statistically significant increase in GI tumors at the low concentrations (5-30 mg Cr(VI)/L water)
  - Water at these levels has poor color and appearance
- NTP results show a lower efficacy of the mouse stomach to reduce Cr(VI); should not apply to humans
  - Detoxifying capacity of gastric environment not infinite
  - At huge doses, the reducing capacity of the mouse GI tract was likely exceeded
- High-dose oral cancers in rats are typical of portal-of-entry effect, before any detoxification may occur

Overview of materials submitted by Dr. Silvio De Flora

EXTRA MATERIALS

Effect of antacids

- Petrilli and De Flora (1982)
  - Subjects treated with cimetidine or ranitidine (inhibitors of histamine H_2 receptors) reduced ~1.5 µg Cr(VI)/mL
  - Untreated subjects: 9.2 µg Cr(VI)/mL
- De Flora et al. (1987)
  - Administration of antacid drug at dinner: elevated pH and no post-dinner peak of Cr(VI) reducing capacity
  - No effect on Cr(VI) reduction due to lunch or breakfast
- Antiulcer/antacid drugs: both inhibit gastric secretion, and neutralize pH
  - Cr(VI) reduction is less efficient, but still occurs

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Discussion Topic 5

Disease states and medical factors

Lead Discussant:
Dr. Kim Barrett

Gastrointestinal physiology and pathophysiology

Relevance for hexavalent chromium absorption and/or reduction

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GI physiology and pathophysiology

- Physiological factors affecting solute absorption
  - Epithelial transport
  - Epithelial barrier function
  - Motility
- Physiological factors affecting solute reduction
  - Gastric acid secretion
  - Gastric emptying
  - Intestinal microbiota
- Modulation of these factors in disease

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Key features of the intestinal epithelium and its function

- Vast surface area
- Imperative to allow nutrient uptake while restricting passage of undesirable substances/microorganisms
- Continual turnover
  - Specialization of cell function
- Lifelong symbiotic relationship with a vast commensal microbiota
- Determinant of luminal fluidity
- Dynamically regulated in health and disease

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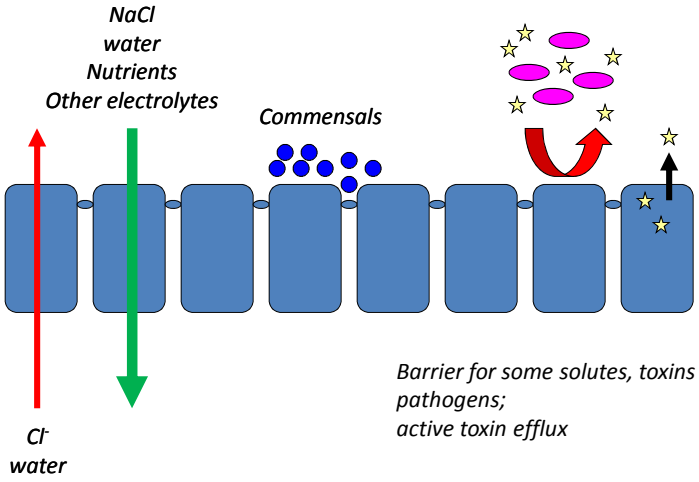
Intestinal epithelial functions



Barrier for some solutes, toxins and pathogens;
active toxin efflux

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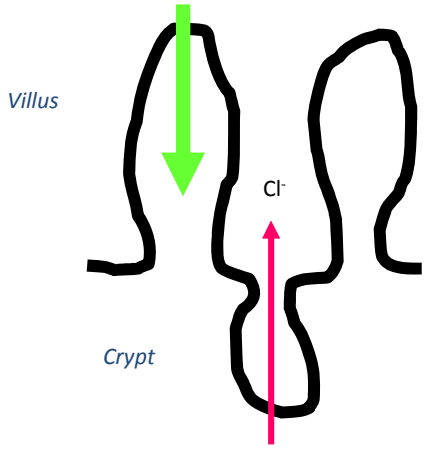
Net small intestinal transport

NaCl, nutrients, other electrolytes (sulfate, Cr?)



- Transport properties of epithelial cells evolve along the crypt-villus axis
- Absorption normally predominates overall but secretion is also ongoing as needed

Segregation of transport events likely an oversimplification

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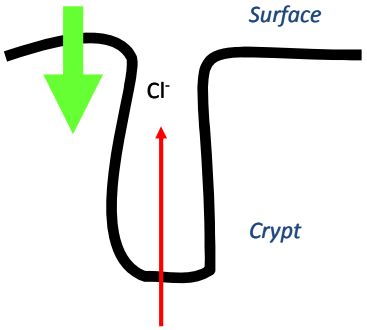
Net colonic transport

Na^+, NaCl
SCFA, other electrolytes



- Water balance in health driven primarily by absorption
- Secretion likely important to modulate local composition of luminal contents
  - Role of reflexes

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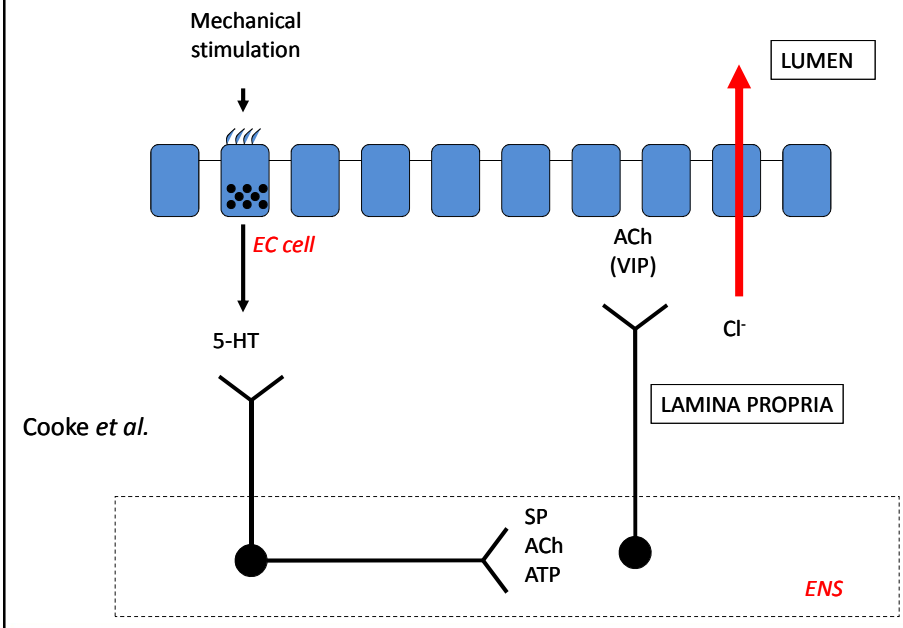

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Intestinal barrier function

- Predominantly ascribable to properties of epithelial tight junctions
  - Claudins, occludin as sealing molecules
  - Cytoplasmic regulatory proteins
  - Perijunctional actomyosin ring
- Distinct junctional properties arise from expression of variable combinations of different sealing and pore-forming claudins
- Permeability decreases distally, and in villus/surface cells *vs.* crypt epithelium

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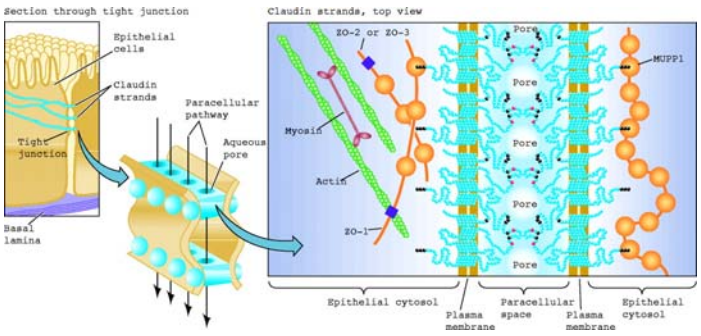
Speculative model of tight junction pores
Tight junctions form as continuous contacts at the apical end of adjacent epithelial cells



Van Itallie, C. M. et al. Physiology 19: 331-338 2004; doi:10.1152/physiol.00027.2004

PHYSIOLOGY

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Effect of claudins on epithelial barrier function

Protein	Resistance	P_{Na}	Tracer flux
Cldn1	↑	-	↓
Cldn2	↓	↑	-
Cldn4	↑	↓	No Δ
Cldn5	↑	↓	No Δ
Cldn7	↑	↑	No Δ
Cldn8	↑	↓	No Δ

Adapted from Van Itallie and Anderson, *Annu. Rev. Physiol.* 68:403-29, 2006

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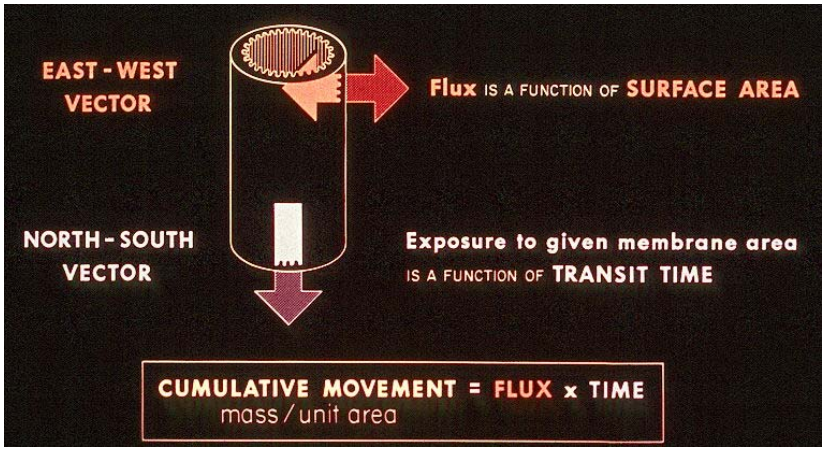
The intestine as an integrator – role of motility in transport regulation



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Modulation of GI transport physiology

- Crypt/villus, cephalocaudal axes
  - Epithelial immaturity/crypt hyperplasia
- Infectious diarrhea, inflammatory bowel diseases
- Functional bowel disorders, constipation
- Medication use
  - Acid suppression
  - Prokinetics
  - Antibiotics (effect on microbiota)
- Fed *vs.* fasted state
- Celiac disease
  - Villous atrophy - loss of absorptive epithelium

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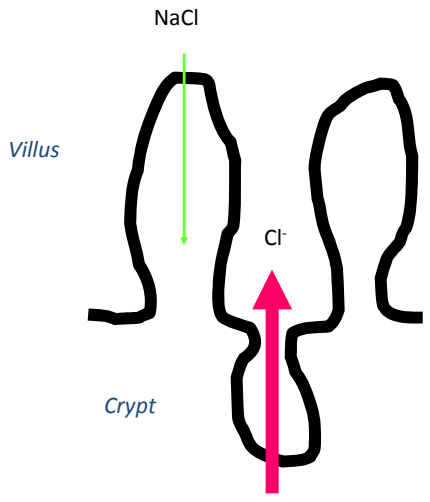
Transport in secretory diarrhea



cAMP induces:

- Reduced NaCl absorption
- Increased secretion
- Net secretion predominates

Motility may also be hastened by distension, further reducing absorption

Note that Na-nutrient absorption usually unaffected

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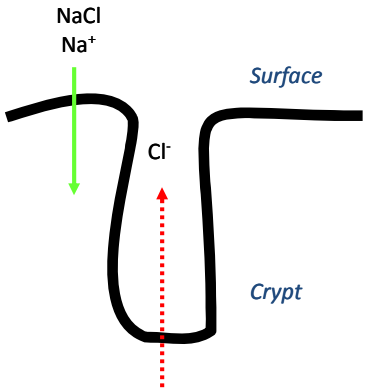
Transport in colonic inflammation or infection



- Secretory capacity largely absent (implications for host defense)
- Markedly reduced electrogenic and/or electroneutral sodium absorption results in fluid accumulation
  - DRA, NHE3, ENaC expression and/or function variably downregulated

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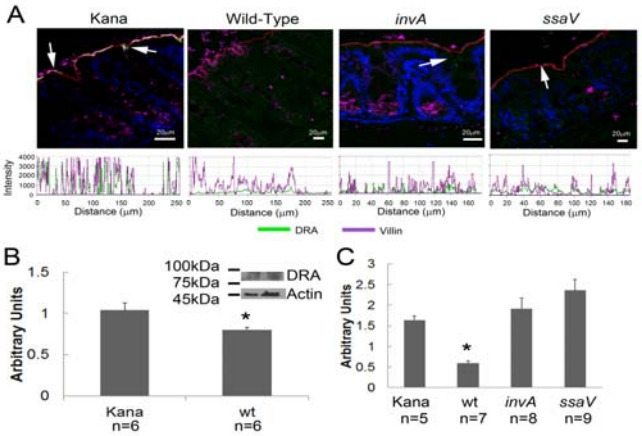
DRA expression in the proximal colon is reduced by wild-type *Salmonella* infection



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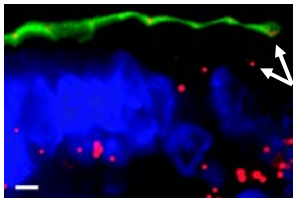
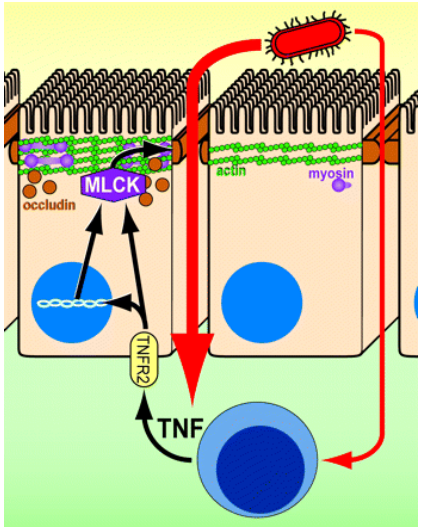
The epithelial barrier in disease

- Reduced barrier function in the setting of inflammation, infection, TPN
- Activation of myosin light chain kinase leads to physical separation of junctional complexes
- Downregulation and/or internalization of some claudins or occludin in response to cytokines and other inflammatory mediators
  - Epithelial immaturity may also contribute
- Upregulation of pore-forming claudin 2
- Ulceration/denudation of epithelium

Barrier dysfunction can be caused by infectious and inflammatory stimuli secondary to occludin endocytosis and actin-myosin contraction





From Turner, J.R., *Am. J. Pathol.* 169:1901-9 (2006)

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Modulation of motility

Intestinal transit slowed by:

- Fed state
- Surgery
- Constipation
- Opioids

Intestinal transit hastened by:

- Excessive distention
- Motilin release
  - During fasting
- Prokinetic agents

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Gastric pH

Reduced by:

- Fasting
- Gastrinoma

Increased by:

- Fed state (buffering despite increased acid secretion)
- Acid-suppressive medications
- Aging
- Pernicious anemia
- Atrophic gastritis (*H. pylori*)

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Gastric emptying

Increased by:

- Fasted state
- Bariatric surgery

Decreased by:

- Presence of nutrients
- Solids *vs.* liquids
- Calories in small intestine
- Gastroparesis
  - Diabetes

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Characteristics of the human microbiota

- A self-organizing community of approximately 10^{14} individual bacteria
  - Possibly as many as 1500 distinct species
  - Cells in average human = 10^{13}
- An integral part of our evolution (and co-evolution)
- A metabolic factory
  - Produces approximately 100x more proteins than the host ("metagenome")
- Bacterial density varies considerably by segment

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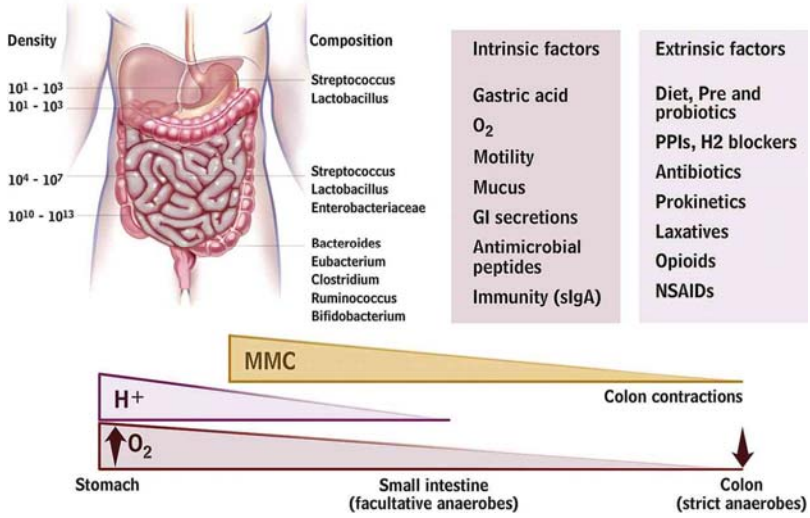

Simren *et al., Gut* doi:10.1136/gutjnl-2012-302167

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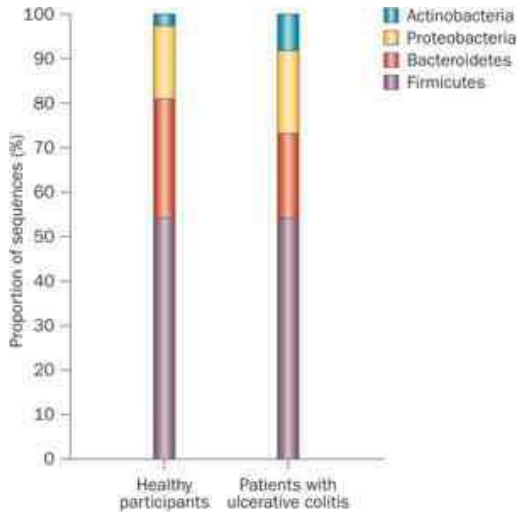
Dysbiosis in disease



Manichanh *et al. Nature Reviews Gastroenterol Hepatol* 9: 599-608, 2012

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Intestinal absorption of Cr(VI)

- In general, the ability to predict hexavalent Cr uptake is limited by a lack of knowledge of the precise route(s) for its absorption
  - As recently as 2011, authors refer to its uptake via sulfate "channels"
  - In fact, there are numerous sulfate *carriers*, including sodium-dependent cotransporters or chloride exchangers, which are members of the Slc13 and Slc26 families of solute carriers
  - Relative contributions of these and/or other carriers and channels to Cr(VI) uptake is unknown, and their precise distribution and/or modulation in disease settings have not been mapped

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Conclusions

- Numerous physiologic conditions as well as disease states have the *potential* to alter uptake of hexavalent chromium
  - Effects on epithelial transport, barrier function, motility, and capacity for reduction of Cr(VI) to Cr(III)
- Our ability to predict the precise impact of these various conditions on chromium assimilation is hampered by a dearth of precise understanding of chromium transport pathways in the gut, as well as their regulation

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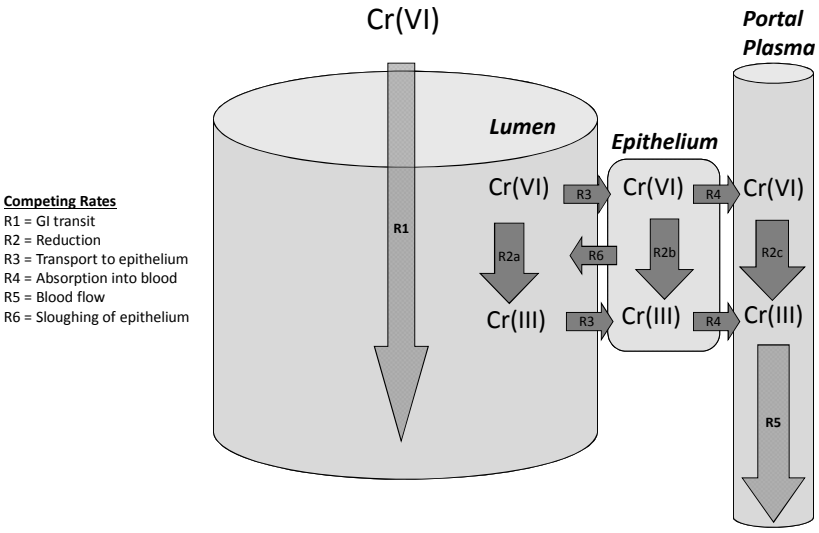
Topic 5

Sean Hays

CrVI Competing Rates



Competing Rates
R1 = GI transit
R2 = Reduction
R3 = Transport to epithelium
R4 = Absorption into blood
R5 = Blood flow
R6 = Sloughing of epithelium

Addressing Disease States & Conditions Quantitatively

	Adults	
Parameter	**Central Tendency**	**Variation**
Gastric Transit Rate (Fasted)	30 min	SD = 22 min (ICRP, 2004)
Gastric Transit Rate (Fed)	90 min	SD = 30 min (ICRP, 2004)
SI Transit Rate	4 hrs	SD = 1.5 hrs (ICRP, 2002)
Absorption Rate	0.00034 L/cm*hr	Range = 2.6-4.3E-4 L/cm*hr (Kirman et al. 2013)
Reduction Rate	44 L/mg*hr	SD = 29 L/mg*hr (Kirman et al. 2013)
Reducing Equivalents (Fasted)	7 mg/L	Range = 4-10 mg/L (Kirman et al. 2013)
Reducing Equivalents (Fed)	30 mg/L	Range = 10-60 mg/L (De Flora et al., 1987)
Stomach Lumen Volume	240 g	CV = 1.1 (ICRP, 2002)
SI Lumen Volume	315 g	CV = 0.69 (ICRP, 2002)
SI Tissue Volume	625 g	SD = 77 (ICRP, 2002)
Body Weight	80 kg	95th %-ile ~120 kg (EFH, 2011)
SI Length	270 cm	Range = 229-337 cm (ICRP, 1975)
Gastric Peak pH (fed)	5	Range = 4-6 (Dressman et al., 1990)
Gastric Baseline pH (fasted)	1.7	Range = 1-2.5 (Dressman et al., 1990)
Time to Return to Baseline	3 hrs	Range 2-4 hrs (Dressman et al., 1990)
SI Lumen pH	6.5	Range 5.5-7.5 (Russell et al., 1993)

Addressing Disease States & Conditions Quantitatively

	Adults		Conditions that:	
Parameter	**Central Tendency**	**Variation**	**Increase Parameter Value**	**Decrease Parameter Value**
Gastric Transit Rate (Fasted)	30 min	SD = 22 min (ICRP, 2004)	Diarrhea	
Gastric Transit Rate (Fed)	90 min	SD = 30 min (ICRP, 2004)	Diarrhea	
SI Transit Rate	4 hrs	SD = 1.5 hrs (ICRP, 2002)	Diarrhea, Excessive distension, Prokinetic Agents	Surgery, Constipation, Opioids
Absorption Rate	0.00034 L/cm*hr	Range = 2.6-4.3E-4 L/cm*hr (Kirman et al. 2013)		
Reduction Rate	44 L/mg*hr	SD = 29 L/mg*hr (Kirman et al. 2013)		
Reducing Equivalents (Fasted)	7 mg/L	Range = 4-10 mg/L (Kirman et al. 2013)		
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Stomach Lumen Volume	240 g	CV = 1.1 (ICRP, 2002)		
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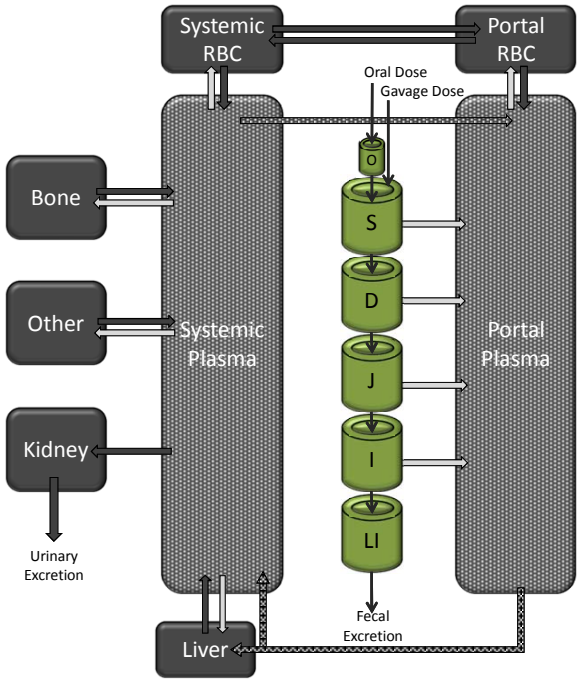
Body Weight	80 kg	95th %-ile ~120 kg (EFH, 2011)		
SI Length	270 cm	Range = 229-337 cm (ICRP, 1975)		
Gastric Peak pH (fed)	5	Range = 4-6 (Dressman et al., 1990)		Gastrinoma
Gastric Baseline pH (fasted)	1.7	Range = 1-2.5 (Dressman et al., 1990)	Acid-Suppressive Medication, Aging, Pernicious Anemia, Atrophic Gastritis (H. pylori)	
Time to Return to Baseline	3 hrs	Range 2-4 hrs (Dressman et al., 1990)		
SI Lumen pH	6.5	Range 5.5-7.5 (Russell et al., 1993)		

Key questions for understanding impact to risk assessment

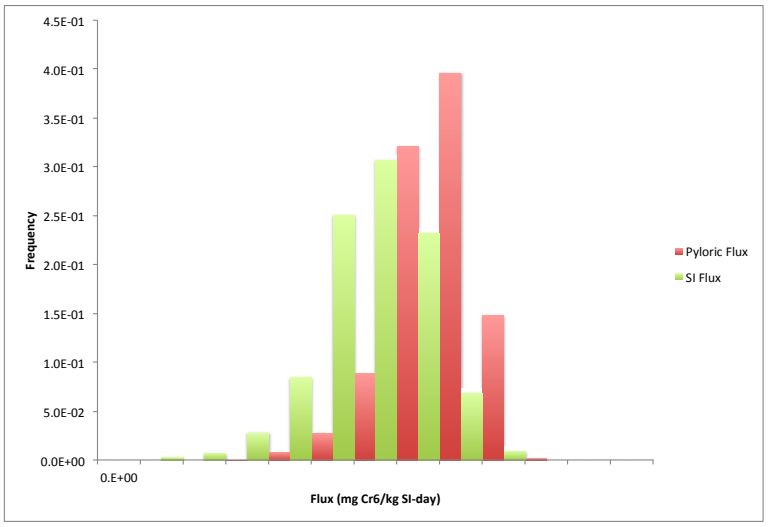
- Does the altered condition/state result in parameter values outside normal variation?
- If so, does the change in parameter value cause an increased dose (CrVI) delivery to SI?

CrVI PBPK Model



Kirman et al. (2013)

Using Variability Information in a Quantitative Manner

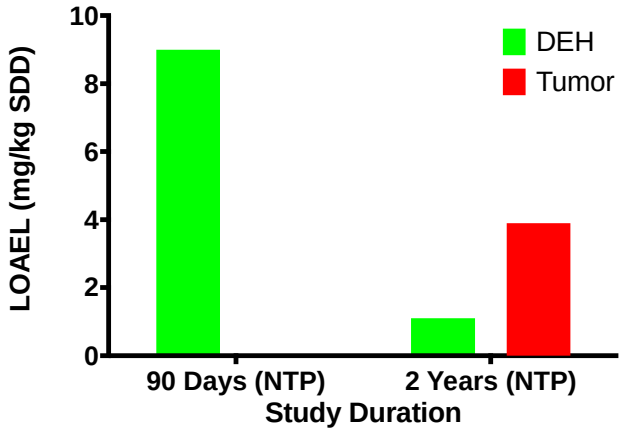


Key questions for understanding impact to risk assessment

- Does the altered condition/state result in parameter values outside normal variation?
- If so, does the change in parameter value cause an increased dose (CrVI) delivery to SI?

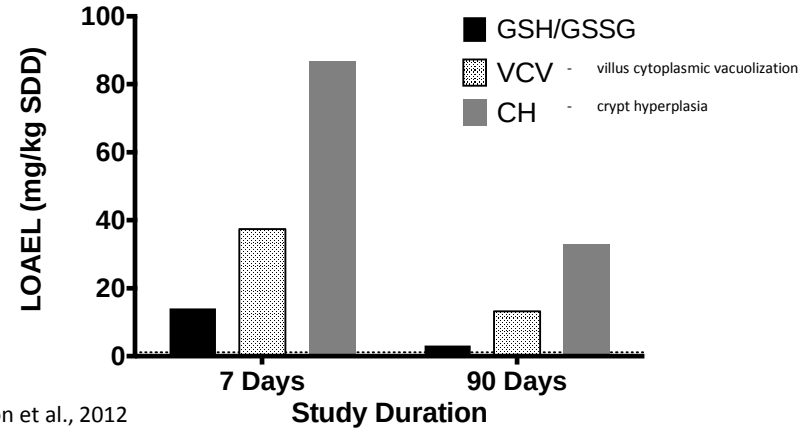
- Is altered condition/state chronic or acute in nature?

PODs vs. Duration



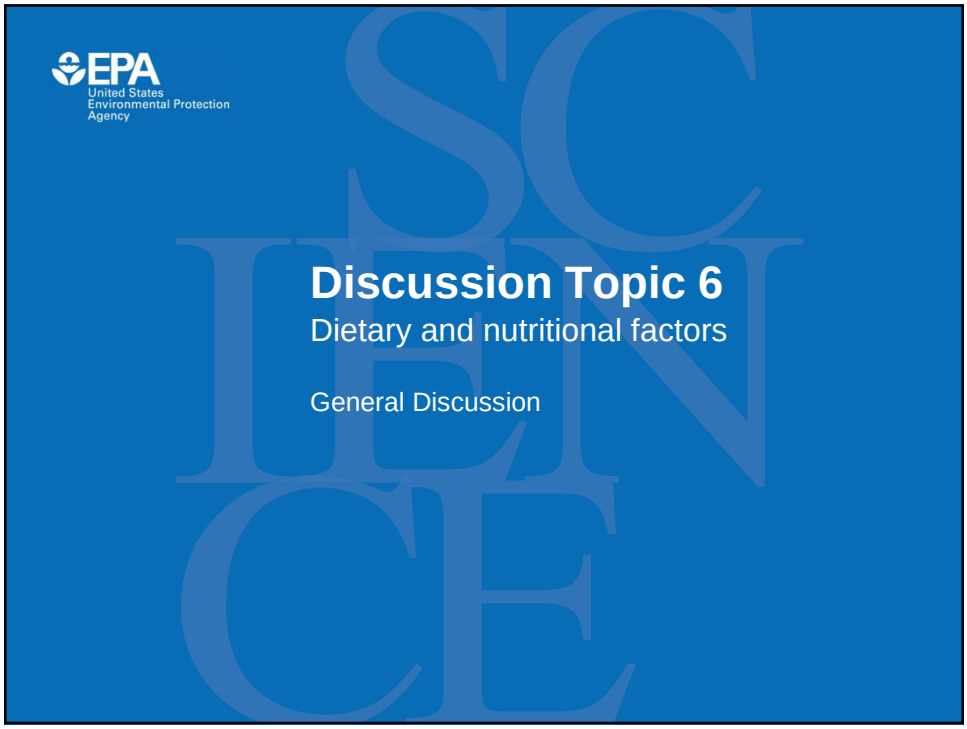
DEH – diffuse epithelial hyperplasia

PODs vs. Duration



Thompson et al., 2012

Depending upon disease state, an acute, subchronic or chronic RfD may be most appropriate for assessing risk.



Topic 6

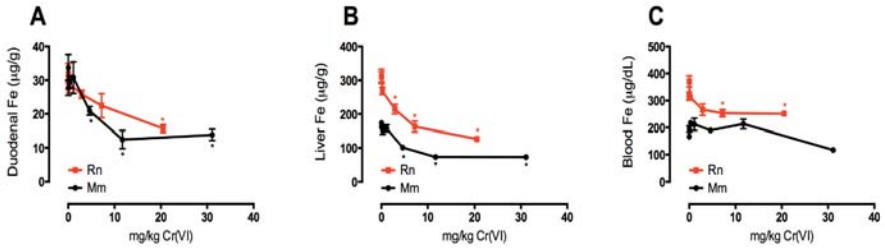
Sean Hays

Effect of High Dose Cr(VI) on Iron Homeostasis

NTP (2008a): Findings of Anemia

- In the 2-year NTP bioassay, microcytic hypochromic anemia reported in male rats and female mice which was more severe in rats
- Anemia ameliorated with time, but significant changes in some hematological markers were still evident in the high dose group rats at one year, which was the final time point that hematology was evaluated
- These anemic responses to high concentrations of Cr(VI) suggest interference with iron (Fe) absorption and/or homeostasis

Effects on Tissue and Serum Iron After 90 Days of Cr(VI) Exposure



Dose-dependent decreases in total Fe in duodenum, liver, and serum in both rats and mice (Thompson et al., 2012)

Iron Metabolism and Cr(VI)

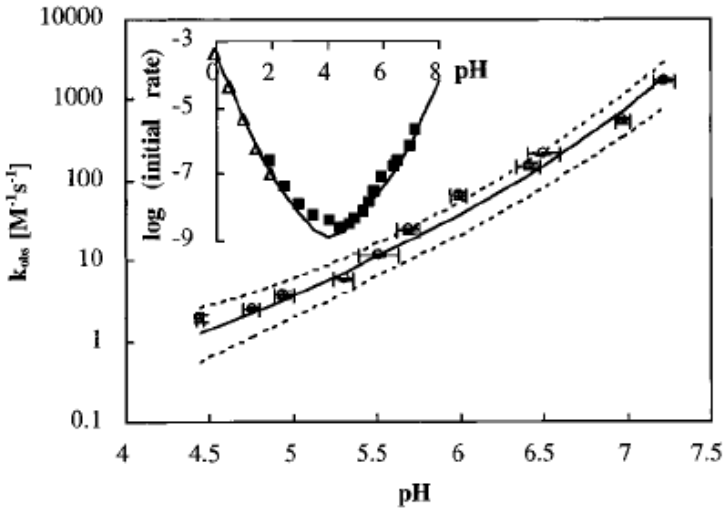
- Cr(VI), Cr(V), and Cr(IV) can oxidize ferrous iron (Fe2+) to ferric iron (Fe3+) (Buerge and Hug, 1997; Fendorf and Li, 1996):
  - Cr(VI) + Fe2+ Cr(V) + Fe3+ (i)
  - Cr(V) + Fe2+ Cr(IV) + Fe3+ (ii)
  - Cr(IV)+ Fe2+ Cr(III) + Fe3+ (iii)
- Fe^{2+} is transported across the duodenum

Iron Metabolism and Cr(VI)

- The major form of non-heme dietary iron is Fe^{3+}, which must be reduced to Fe^{2+} prior to absorption by enterocytes in the proximal small intestine
- Anemic effects were not observed in rodents chronically exposed to Cr(III) (NTP, 2008b; Stout et al., 2009)
- Hypothesis: High Cr(VI) concentrations in the lumen of the small intestine oxidize luminal Fe^{2+} perturbing iron (Fe) absorption

Cr(VI) Reduction by Fe(II):

pH Dependence (Buerge 1997)



EPA United States Environmental Protection Agency

Discussion Topic 7

Lifestages

General discussion

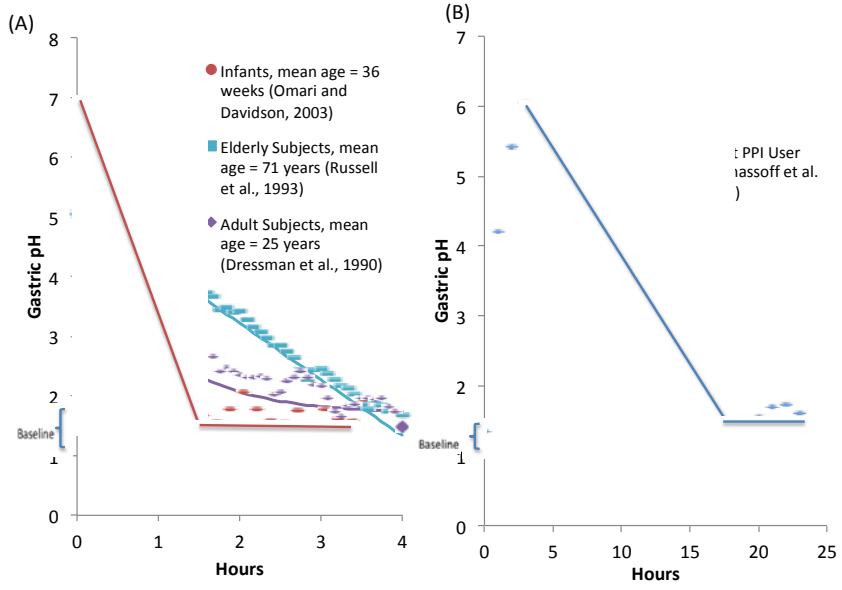
Topic 7

Sean Hays

Addressing Life Stage Differences

Parameter	Neonate	Infant/Child	Youth	Adult	Elderly	Reference
Age	0-0.25 yr	0.25-6 yr	6-18 yr	18-60 yr	60-75 yr	NA
Exposure Duration (years)	0.25	5.73	12	42	15	Professional judgement
Body Weight (kg)	5.5	15.2	48.9	80	80	EPA (2011)
Cr Exposure Events per Day	4 (2 fed; 2 fasted)	4 (2 fed; 2 fasted)	6 (3 fed; 3 fasted)	6 (3 fed; 3 fasted)	6 (3 fed; 3 fasted)	Barraj et al. (2009; split assumed
Number of Meals/Day	8	4	3	3	3	Professional judgement
Peak Gastric Lumen pH (fed)	7	7	6	5	5	Dressman et al. (1990); Russell et al. (1993); Nagita et al. (1996); Omari and Davidson 2003
Baseline Gastric Lumen pH (fasted)	3.5	2	1.5	1.7	1.3	
Time to return to baseline pH from peak (hr)	2	2	3	3	4	
Return to baseline behavior	Linear	Linear	Nonlinear	Nonlinear	Linear	
Gastric Lumen Reducing Equivalents, fed (mg/L)	30	30	30	30	30	Adult value based on DeFlora et al. (1987); value adopted for other age groups
Gastric Lumen Reducing Equivalents, fasted state (mg/L)	7	7	7	7	7	Adult value based on Kirman et al. (2013); value adopted for other age groups
Gastric Lumen Transit, Fed (hours)	1.25	1.17	1.17	1.38	1.38	ICRP (2002)
Gastric Lumen Transit, Fasted (hours)	0.17	0.50	0.50	0.50	0.50	
Small Intestines Lumen Transit (hours)	4	4	4	4	4	
Small Intestines Lumen pH	6.5	6.5	6.5	6.5	7	Kararli (1995); Russell et al. (1990)
SI Length (cm)	120	150	220	270	270	ICRP (2002)
SI Mass (fbw)	0.015	0.014	0.0076	0.0078	0.0078	
SI Lumen Mass (fbw)	0.136	0.0069	0.0033	0.0039	0.0039	
Stom Mass (fbw)	0.0025	0.0023	0.0017	0.0018	0.0018	
Stom Lumen Mass (fbw)	0.0098	0.0049	0.0024	0.0030	0.0030	

Model Application to Humans: Age Differences in Gastric pH



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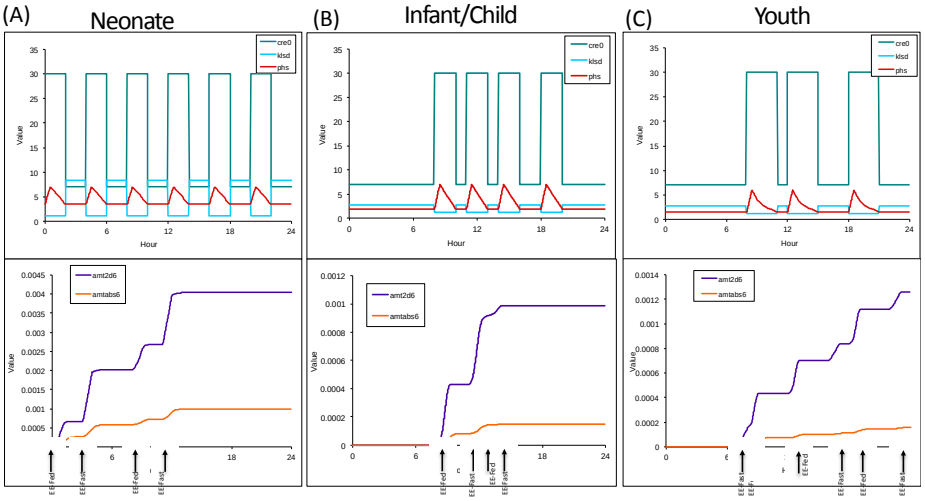
Model Application to Humans: Estimating Internal Dose to Different Age Groups



EE-Fast = exposure event, fasted state; EE-Fed = exposure event, fed state

CREO = Initial concentration of gastric reducing equivalents; KLSD = gastric transit rate constant

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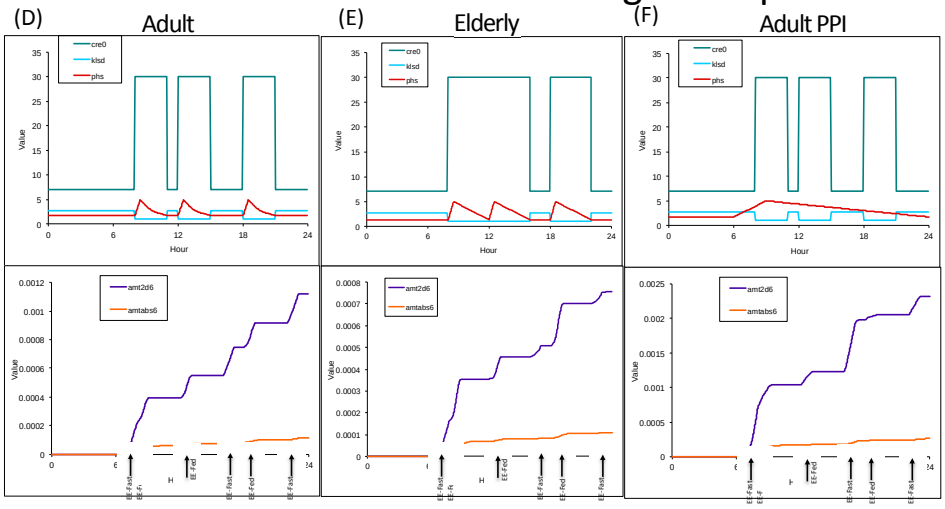
Model Application to Humans: Estimating Internal Dose to Different Age Groups



EE-Fast = exposure event, fasted state; EE-Fed = exposure event, fed state

CREO = Initial concentration of gastric reducing equivalents; KLSD = gastric transit rate constant

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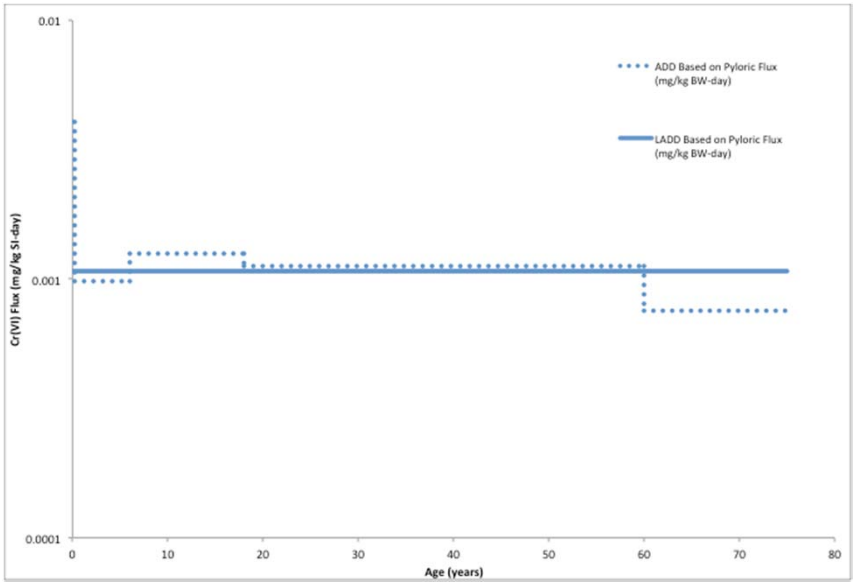
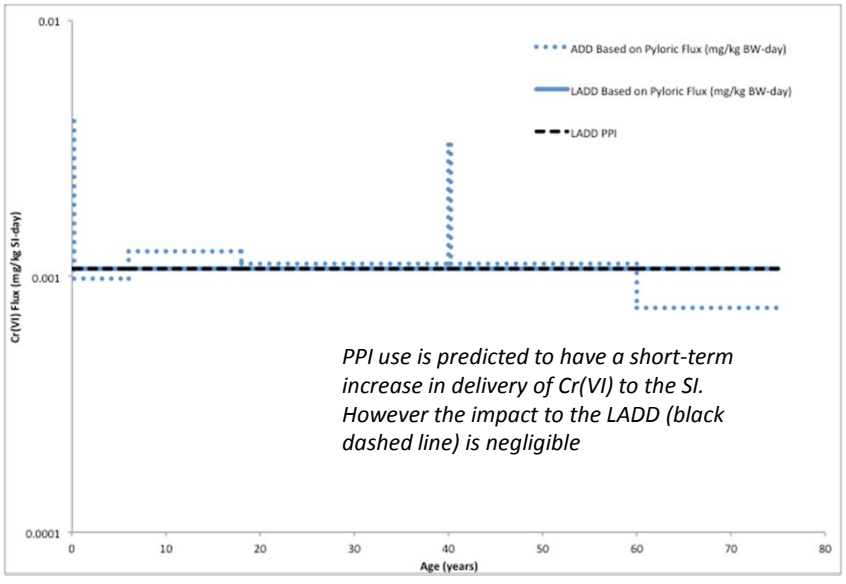
Contribution of Age Group-Specific Doses to Lifetime Average Daily Dose (LADD)



Impact of 3-month PPI Use by Adult



PPI use is predicted to have a short-term increase in delivery of Cr(VI) to the SI. However the impact to the LADD (black dashed line) is negligible