Science Question 6: Relation Between Anemia and Oral Tumors in Rats

Key Points

1. **Mode of Action for Cr(VI) induced oral tumors is not known**
2. **New OECD 488, GLP-compliant Big Blue transgenic rat mutation study is being conducted to examine Cr(VI) mutagenicity in the target rat oral tissues [EPRI Funded]**
3. **Study will be completed this fall**

Deborah Proctor

ToxStrategies, Inc.

June 25, 2014

Transgenic Mutation Study in Big Blue F344 Rats

- **Funded by EPRI Research Contract, ToxStrategies and BioReliance are currently conducting OECD 488, GLP-compliant transgenic mutation assays in Big Blue F344 rats**
- **Study Objective: Examine the mutagenicity of Cr(VI) in the rat oral mucosa to inform the carcinogenicity of Cr(VI)**
- **The Big Blue study is being conducted in 3 phases**
  - DNA extraction from target tissue
  - Positive Control using 4-Nitroquinoline-N-oxide (4NQO)
  - Cr(VI) dosing at carcinogenic dose

Phase 1: DNA Collection From Target Tissue (Origin of Oral Tumors in Rats)

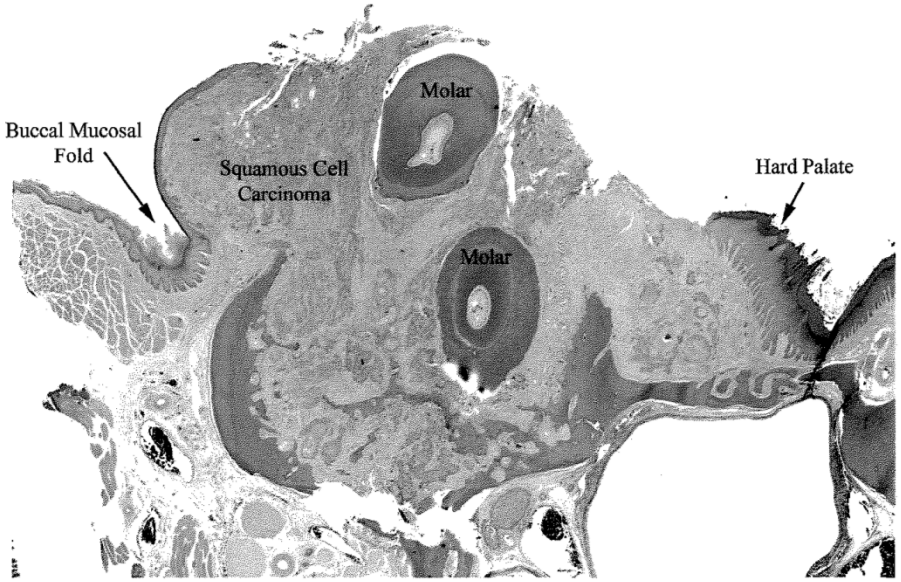

Fig. 1 - Squamous cell carcinoma surrounding a molar tooth. Note location of buccal mucosal fold and degree of keratinization relative to that of the hard palate mucosa.

- Oral mucosa is not conventional tissue for transgenic animal studies
- Target the oral tissues where tumors arose to assess increase in mutation frequency
- "Most of the squamous cell carcinomas … appeared to arise from oral mucosa surrounding the molar teeth (**gingival epithelium**) and/or from the region of the buccal mucosal fold (above the maxillary molars or below the mandibular molars) (Dr. Phil Long, personal communication).

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Phase 2 and 3:
Preliminary Mutation Results for Gingiva/Buccal Fold

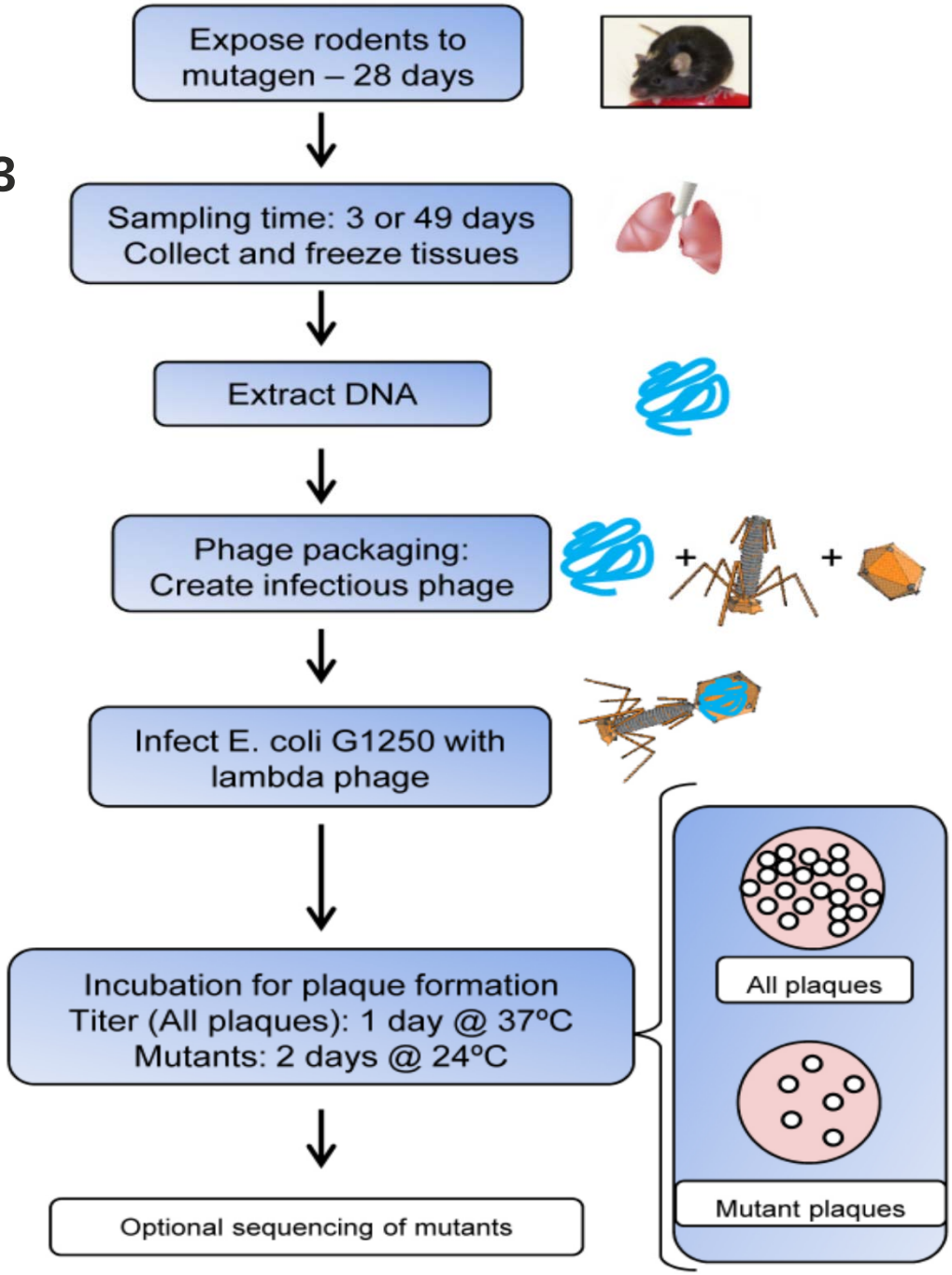
F344 Big Blue Rats OECD 488 Study

Phase	Study	Status	Animals	Mutation Frequency*
2	Control	Completed	5	$62.7 \pm 29.5 \times 10^{-6}$
2	10 ppm 4NQO	Completed	5	$1057.5 \pm 78.0 \times 10^{-6}$
3	Control	Start 7/29/14	5	TBD
3	520 mg/L SDD**	Start 7/29/14	5	TBD

*Preliminary result from ~half samples
4NQO = 4-nitroquinoline-N-oxide; SDD = sodium dichromate dihydrate (Cr6); TBD = to be determined
**DNA from 4NQO study will be packaged again at same time as SDD to serve as positive control

Manuscript will be prepared and submitted in Fall 2014

OECD 488 Study Design Phase 2 and 3



Science Question 6: Relation Between Anemia and Oral Tumors in Rats

Key Points

1. **Anemia is observed at high doses in all of the NTP studies of Cr(VI); hypothesize that oxidation of Fe^{2+} disturbs intestinal absorption**
2. **Multiple lines of evidence support disturbance in Fe homeostasis with Cr(VI) exposures**
3. **Although anemia is associated with several outcomes observed in the NTP chronic bioassay, the association with oral cancer is not proven**
4. **Several etiological factors are likely involved for Cr(VI) carcinogenesis in rat oral cavity**

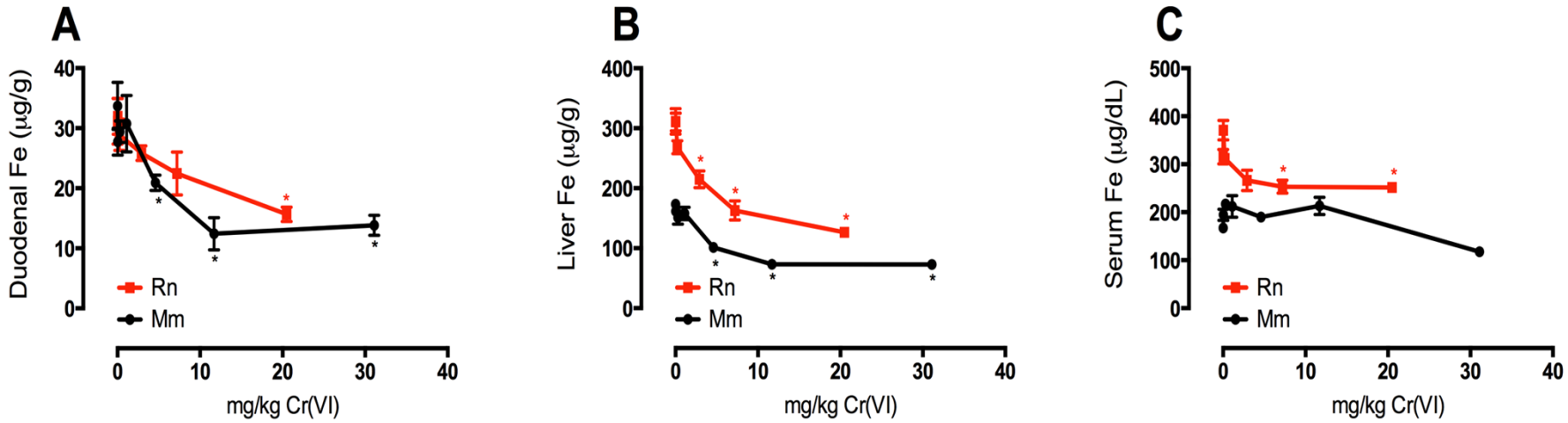
Mina Suh

ToxStrategies, Inc.

June 25, 2014

ToxStrategies

Effects of Cr(VI) on Total Fe in the 90 Day Study



Source: Suh et al. (2014); drinking water exposures of 0.1 to 180 mg CrVI/L

- **Dose-dependent decreases in Fe levels in the duodenum, liver, and blood of rats and mice**
- **Decreased liver Fe is more severe in rats than mice**

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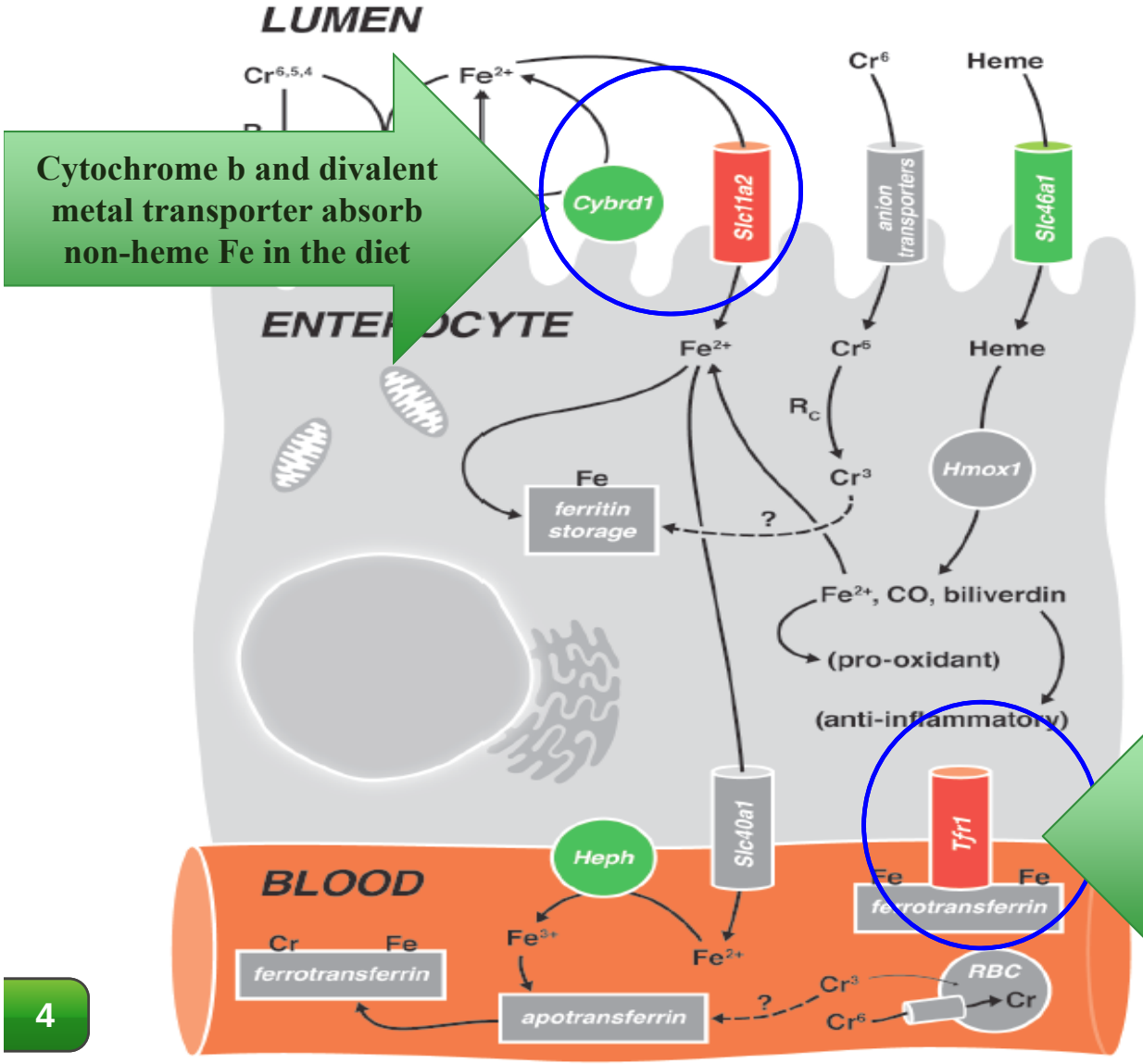
Iron Content of Bone Marrow of Rats After 90 Days of Cr(VI) Exposure

Iron Content[1]	Number of Animals[2]					
	0 mg/L	0.1 mg/L	1.4 mg/L	20 mg/L	60 mg/L	180 mg/L
Slight	0	0	0	0	0	5
Moderate to Slight	1	0	0	1	3	0
Moderate	4	5	5	4	2	0

[1] Slight indicates low Fe content
[2] 5 rats per dose group were evaluated

- **Low levels of Fe in bone marrow of the high dose rats**
- **Fe content of mouse bone marrow was not affected**

Cr(VI) Induces Changes in Expression of Genes Associated with Fe Homeostasis



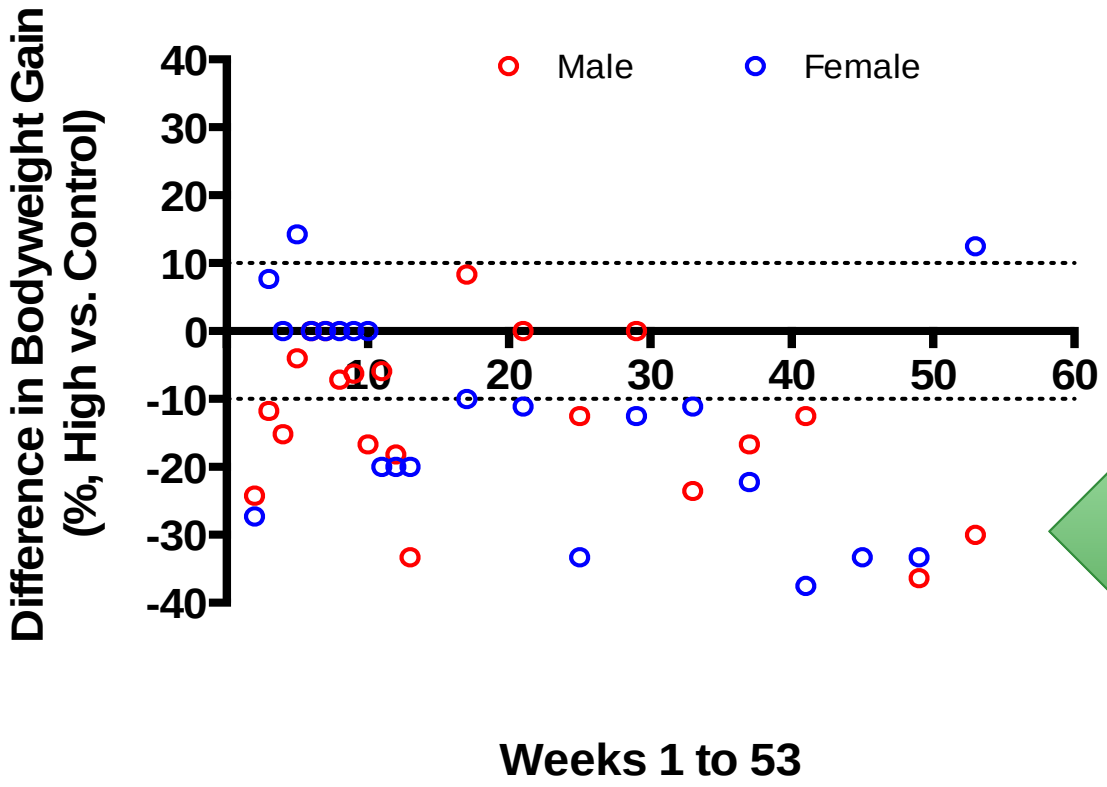
Cytochrome b and divalent metal transporter absorb non-heme Fe in the diet

Changes consistent with rats on Fe-deficient diet (Collins et al. 2005)

Transferrin receptor 1 is involved in Fe uptake from blood

Source: Suh et al. 2014

Percent Difference in Rat Bodyweight Gain in 180 mg/L vs. Controls (NTP, 2008)



24 of 44 intervals exceeded 10% decrement in bodyweight gain

- **Doses that cause ≥10% decreases in bodyweight gain (not absolute bw) may exceed the maximally tolerated dose (Eaton and Klassen, 2001; FDA, 2008; OECD 2008; Rhomberg et al. 2007)**

Effect of Cr(VI) on Water Intake in Rats (NTP, 2008)

Percent Difference from Controls for Water Intake Adjusted by Bodyweight[a]

Males

Cr(VI) (mg/L)	Weeks 1-13	14-52	53-101
5	1.9%	0.5%	1.6%
20	-1.3%	-0.4%	1.6%
60	-13.7%	-10.9%	-11.6%
180	-17.1%	-13.2%	-14.9%

Females

SDD (mg/L)	Weeks 1-13	14-52	53-101
5	-0.5%	0.4%	-0.8%
20	-3.6%	-1.8%	-5.3%
60	-18.1%	-12.4%	-18.9%
180	-25.5%	-18.5%	-18.7%

- **Intake reduced as much as 26% in the 180 mg/L dose groups**
- **Not dehydrated but decreased intake can reduce salivary output**
- **Saliva is recognized to possess anti-carcinogenic properties**

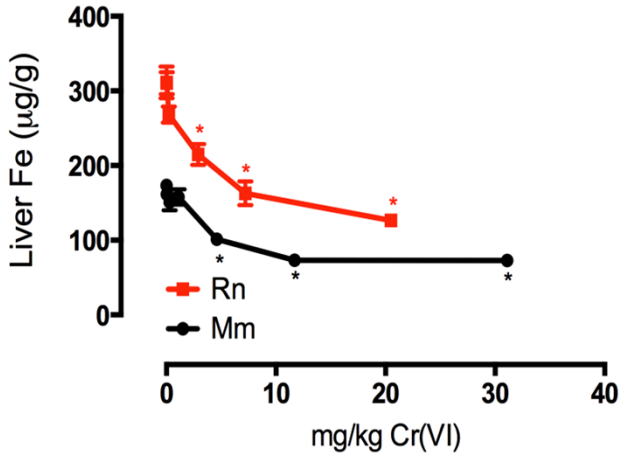
[a] Water intake adjusted by bodyweight (mL/g) was calculated by dividing the daily water consumption by bodyweight.

Summary of Effects Potentially Associated with Fe Homeostasis in NTP (2008)

Endpoint Associated with Cr(VI) Exposure in NTP Chronic Bioassay	Dose-related trends
Histiocytic infiltration (Liver, duodenum, mesenteric lymph nodes)	Rats: Increased for all 3 tissues Mice: Liver of females only
Fatty liver	Rats (F) only
Chronic liver inflammation	Rats (F) only
Salivary gland atrophy	Rats (F) only
Oral squamous cell carcinoma	Rats (M and F)

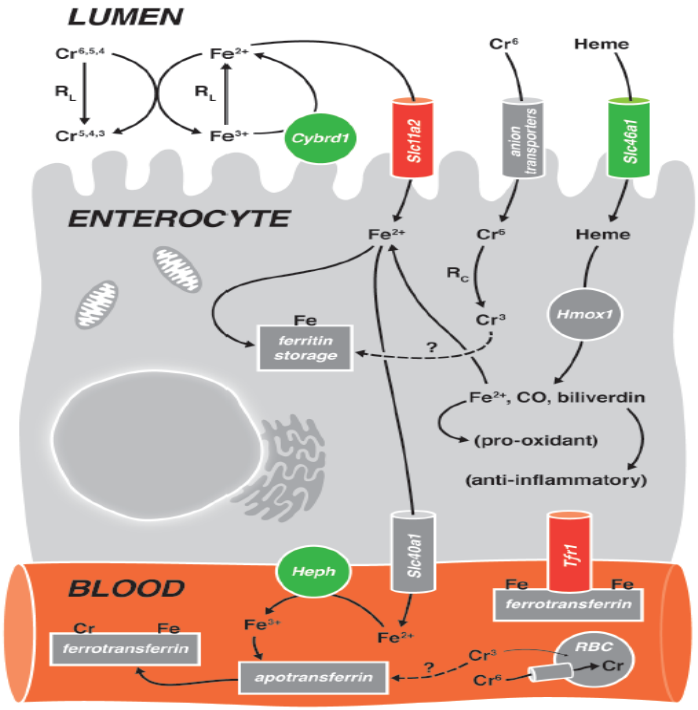
- **Not attempting to support that all effects in the Cr(VI) NTP study were due to disruption of Fe status**
- **In general, these effects are more pronounced in females than males and in rats than mice**
- **Link between anemia and oral cancer remains to be proven**

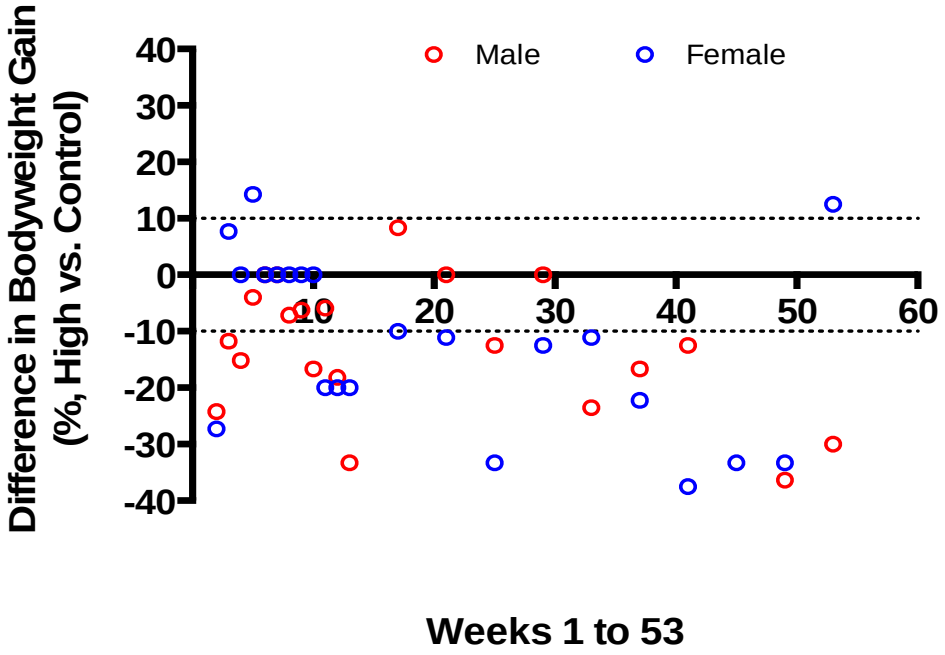
Summary of the Data



Percent Difference from Controls for Water Intake Adjusted by Bodyweight

Females SDD (mg/L)	Weeks 1-13	14-52	53-101
5	-0.5%	0.4%	-0.8%
20	-3.6%	-1.8%	-5.3%
60	-18.1%	-12.4%	-18.9%
180	-25.5%	-18.5%	-18.7%





Science Question 6: Relation Between Anemia and Oral Tumors in Rats

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Supported by ACC
June 25, 2014

ToxStrategies

Oral Tumor Response In F344 Rats (NTP, 2008)

	0 mg/L	14 mg/L	57 mg/L	172 mg/L	516 mg/L
Males					
Mean survival days	695	670	672	692	694
Oral carcinoma	0/50	0/50	0/49	0/50	6/49*
Females					
Mean survival days	696	691	694	686	685
Oral carcinoma	0/50	0/50	0/50	2/50[a]	11/50**

[a] Exceeded historical control range for drinking water studies and for all routes of administration; *p ≤ 0.05, **p ≤ 0.001 compared to concurrent control by poly-3 test

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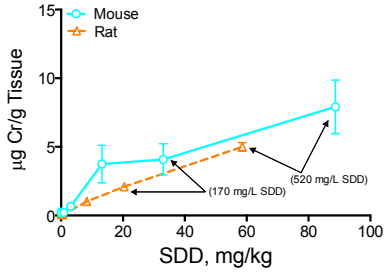
Data Do Not Support POE Effects

- Cr levels in upper palates of rats and mice were comparable (slightly higher in mice; Thompson et al., 2012)



- No coherent dose-dependent changes in gene expression palates of rats and mice (unpublished data)
- Cr levels in palates never reached levels that elicited gene responses in duodena (i.e. ≤ 10 µg/g)