

# Comments on Science Question #3: Gastric Reduction of CrVI



## Take Home Message

- New gastric reduction data from fed & fasted human samples provide
  - Evidence for multiple pools of reducing agents in humans
  - More data for pH dependence of gastric reduction of CrVI

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# Outstanding Issues - Gastric Reduction

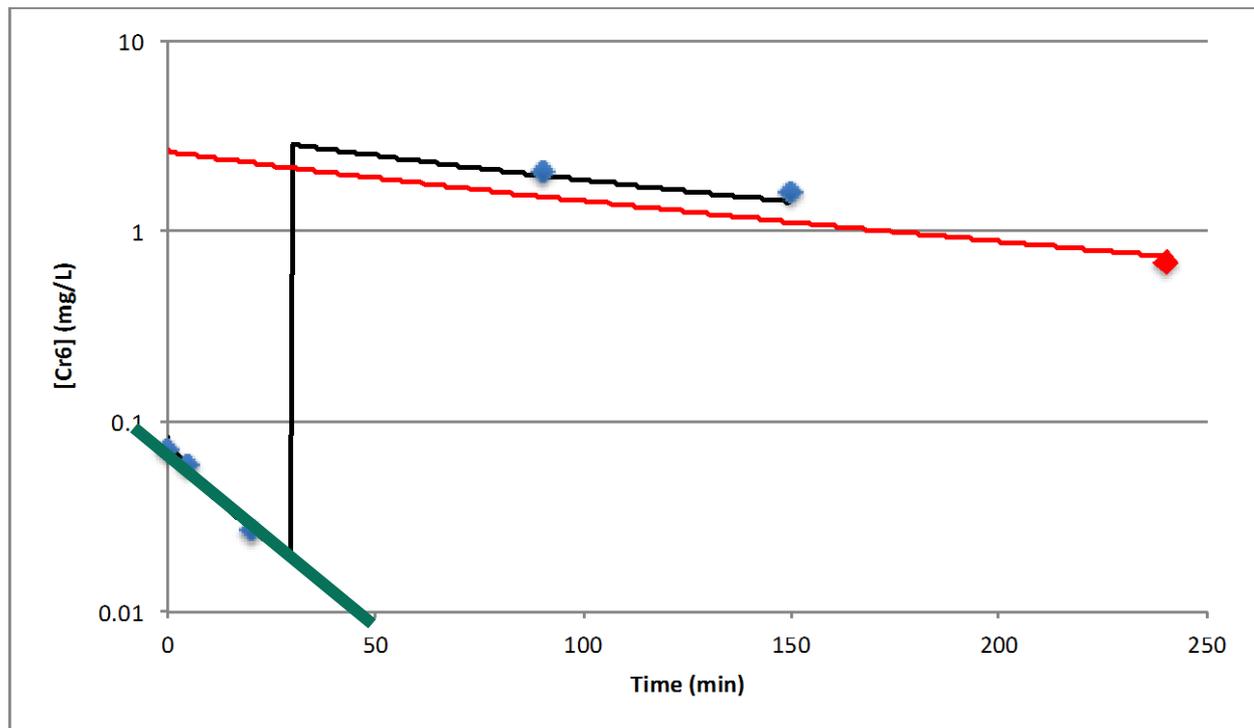
- # of distinct pools of reducing agents
- Effect of dilution
- pH dependence
- Fed vs fasted state



# Ex Vivo Study Design

- Fed and fasted gastric samples obtained from 7 individuals (obtained from Dr. Silvio de Flora)
- In order to provide information on multiple pools, we've modified the study protocol to include a split spike of Cr(VI)
  - *Estimate sample capacity (single spike ~3 mg/L)*
    - Measure Cr(VI) at 240 minutes
  - *Split spike run*
    - 1st Cr(VI) spike at time=0: ~0.1 mg/L to characterize rate & capacity at low concentrations; Measure Cr(VI) at 0.25, 5, 20 minutes
    - 2nd Cr(VI) spike at time=30min: ~3 mg/L to characterize rate & capacity at high concentrations; Measure Cr(VI) at times 90, 150 minutes

*Split-spike sample approach is yielding very useful data (data for volunteer 14 shown): Black line/Blue Diamonds = Split spike run; Red line/diamond = capacity run*



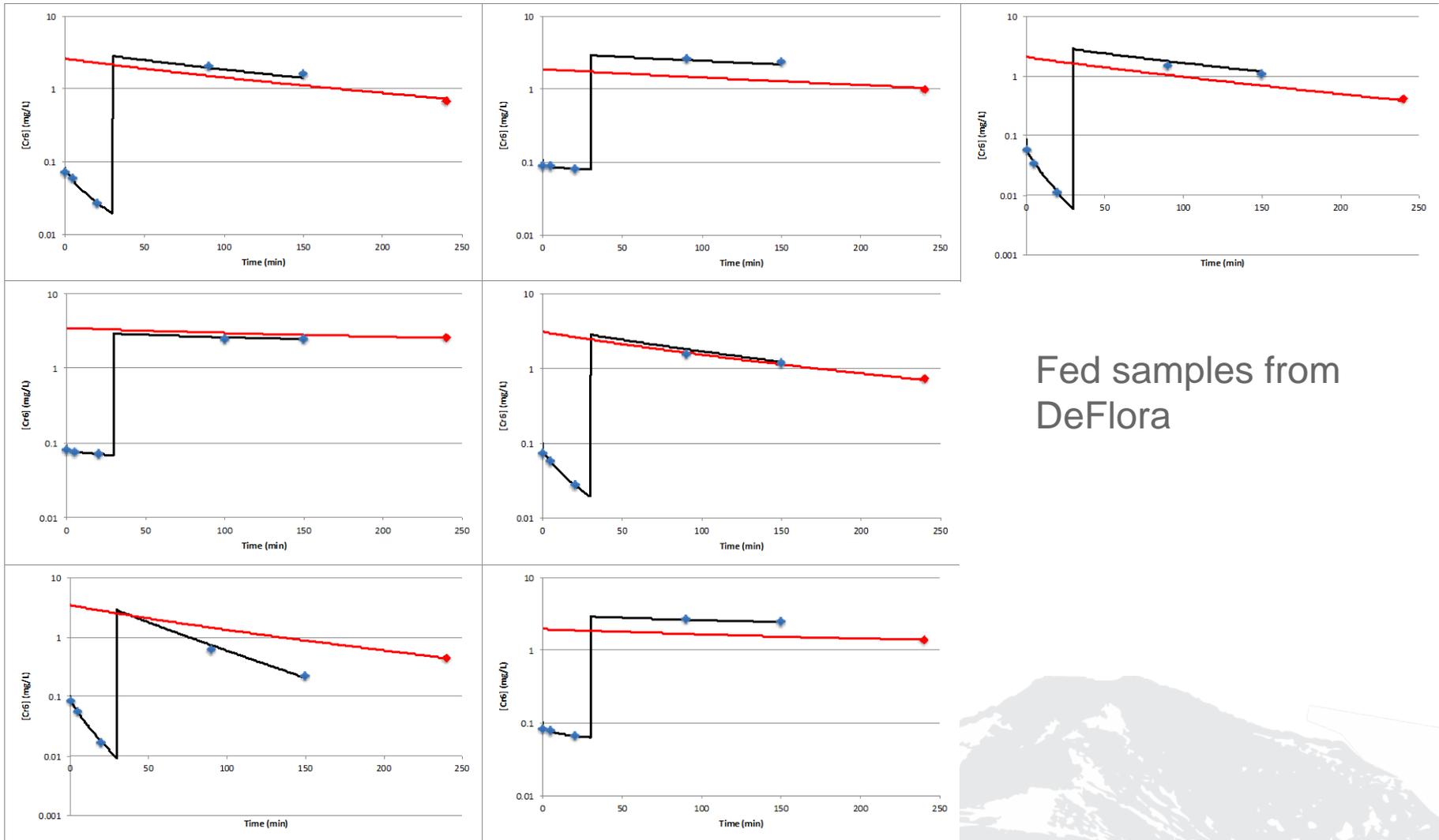
- The slopes of the curves for time points <30 minutes are much different than for time points >30 min.*
- Low concentrations of Cr(VI) are more rapidly detoxified than high concentrations*

These results are consistent with the presence of at least 2 pools:

- 1) Fast reaction rate with small capacity (<1 mg/L)
- 2) Slow reaction rate with large capacity (>10 mg/L)

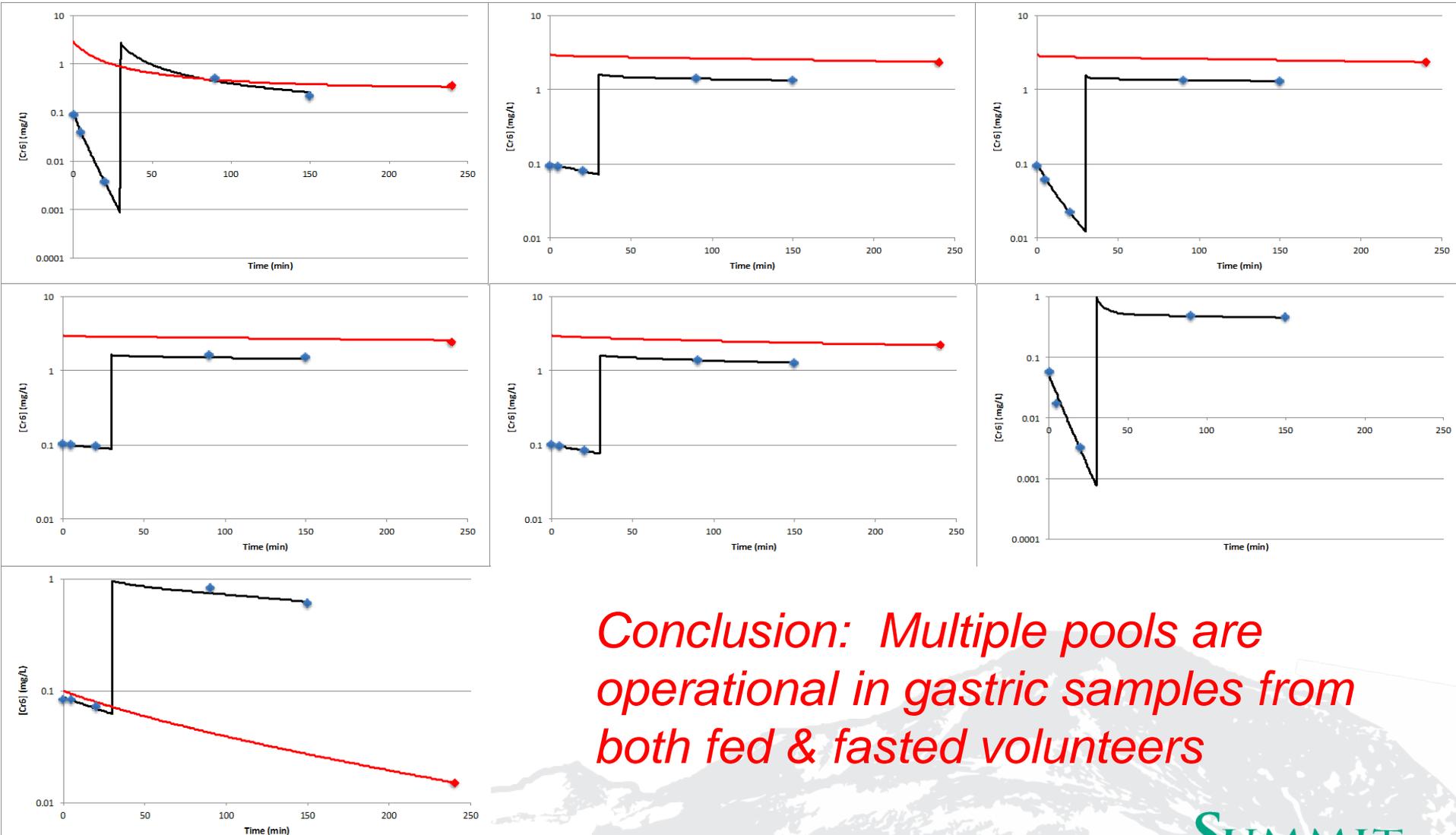
Without the split spike, the fast rate would not be easily detected and the slower/higher capacity reducing pool gets lost due to detection limit issues

# Observed Reduction for Fed Samples



Fed samples from DeFlora

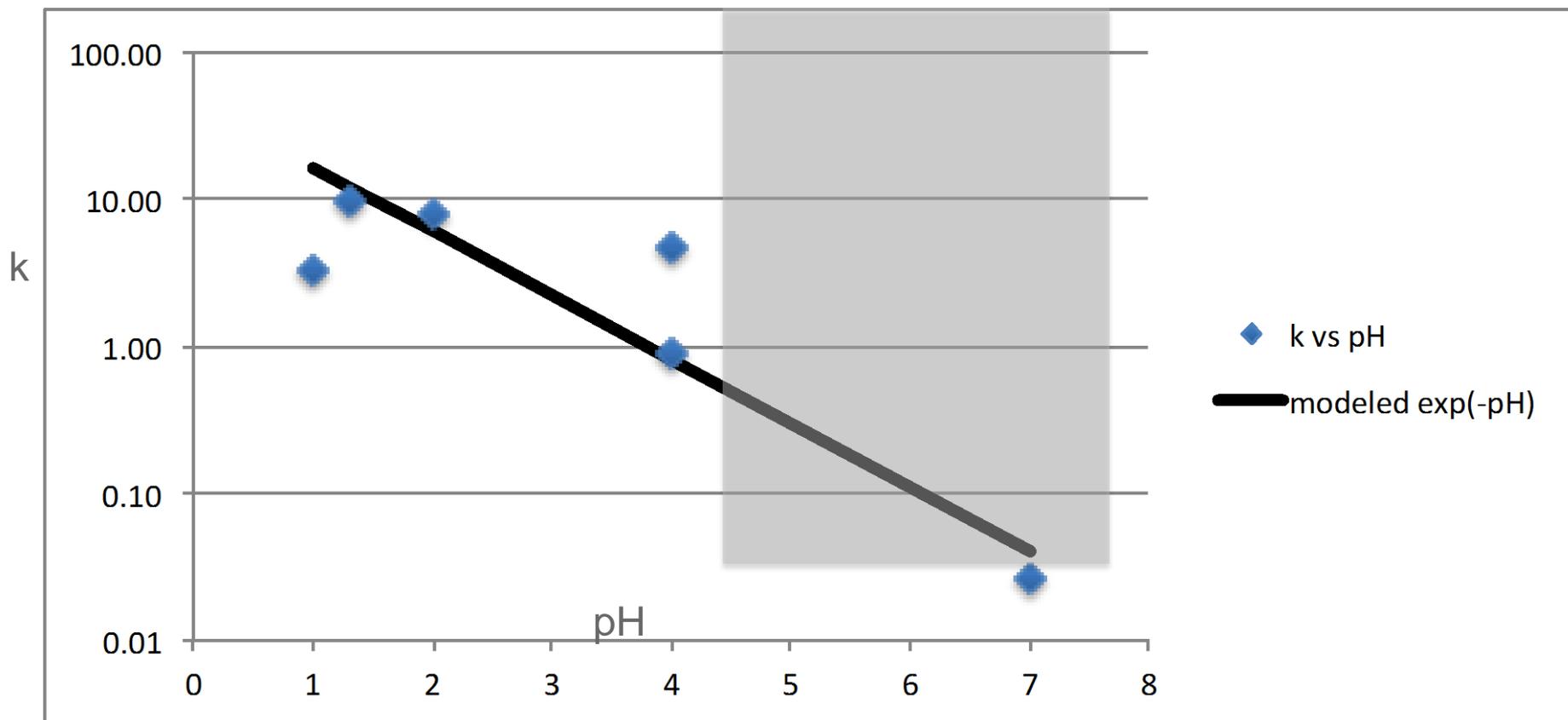
# Similar Observed Reduction for Fasted Samples



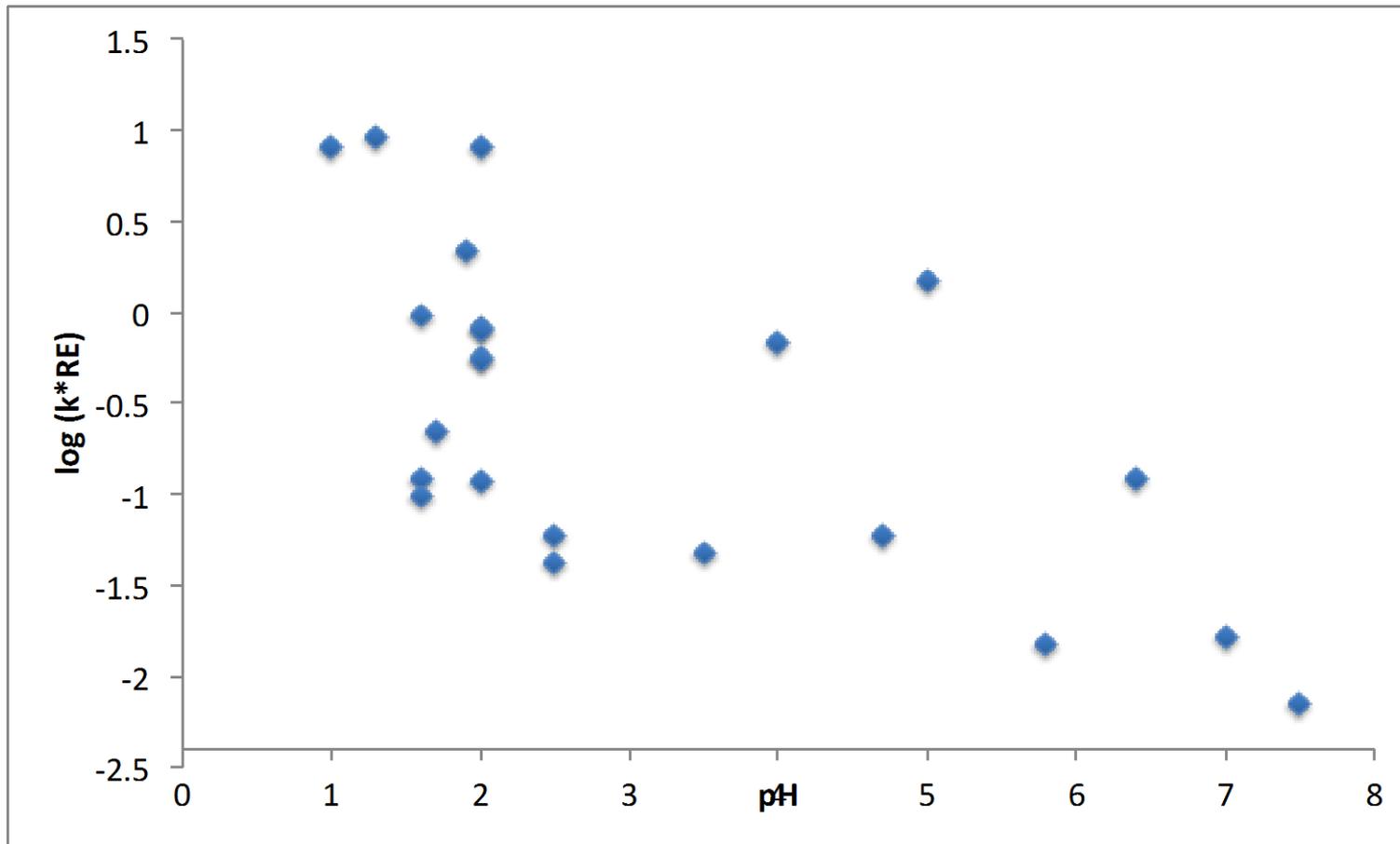
*Conclusion: Multiple pools are operational in gastric samples from both fed & fasted volunteers*

# Our Previous Results, Limited pH Coverage

New data provide more complete coverage of the range of pH values



# pH Dependence



*pH-dependency is well covered between pH 1 to pH 7.5*  
*There is considerable variation in effective k*  
*No clear evidence of an inflection point*

# Ex Vivo Reduction Studies: *What have we learned?*

- Dilution impacts reducing equivalents (RE) but not  $k$
- Multiple reduction pools (at least 2) are present in fed & fasted human gastric samples
- There is considerable variation in the pH dependency across samples
- No clear evidence of an inflection point in the relationship between pH & reduction rate
- A manuscript for this data is in preparation and will be submitted for publication in 4<sup>th</sup> Q, 2014.



# Toxicokinetic Threshold

- Science Question #3 refers the concept of a “toxicokinetic threshold”
- This term is not specifically used in Thompson et al. (2013)
- Depletion of gastric reducing equivalents in mice under the conditions of the NTP bioassay may be an important source of nonlinear toxicokinetics to consider in the risk assessment.
- Based on empirical data as well as the PBPK modeling results, depletion of gastric reducing agents in mice under the conditions of the NTP bioassay can result in higher concentrations of CrVI reaching the small intestines.

# Toxicokinetic Threshold

- Predicted depletion of gastric reducing equivalents in mice under the conditions of the NTP bioassay for CrVI (0, 1.4, 5, 21, 60, 180 mg/L)
- Schlosser and Sasso (2014; Table 2), 3-pool model
  - *Pool 1 (fast): depletion ~ 5 mg/L*
    - net reduction rate becomes ~2.5x slower
  - *Pool 2 (slow): depletion ~ 60 mg/L*
    - Net reduction rate becomes 265x slower
  - *Pool 3 (very slow): does not deplete*
- Proctor et al. (2012), 1-pool model
  - *Pool 1: depletion ~21 mg/L (between the depletion points for 2 pools above)*
    - Net reduction rate becomes 0
- Both models predict depletion of RE pool(s) to occur under conditions of NTP bioassay

# Summary

- We agree that accurately modeling gastric reduction of CrVI is key to risk assessment
  - *Mixed second order*
  - *RE dilution dependent, k dilution independent*
  - *Multiple pools of reducing agents*
  - *pH dependence*
- PBPK model is required to model competing rates that govern delivery of CrVI to target tissue (SI)