Comments on Science Question #3: Toxicokinetic Considerations for Dose-Response

Overview:

- Review of Schlosser and Sasso (2014) CrVI Reduction Model
- Use of TK Data from Nonphysiologic Exposure Studies

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EPA Bi-Monthly Meeting
October 30, 2014

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Review of Schlosser and Sasso (2014)

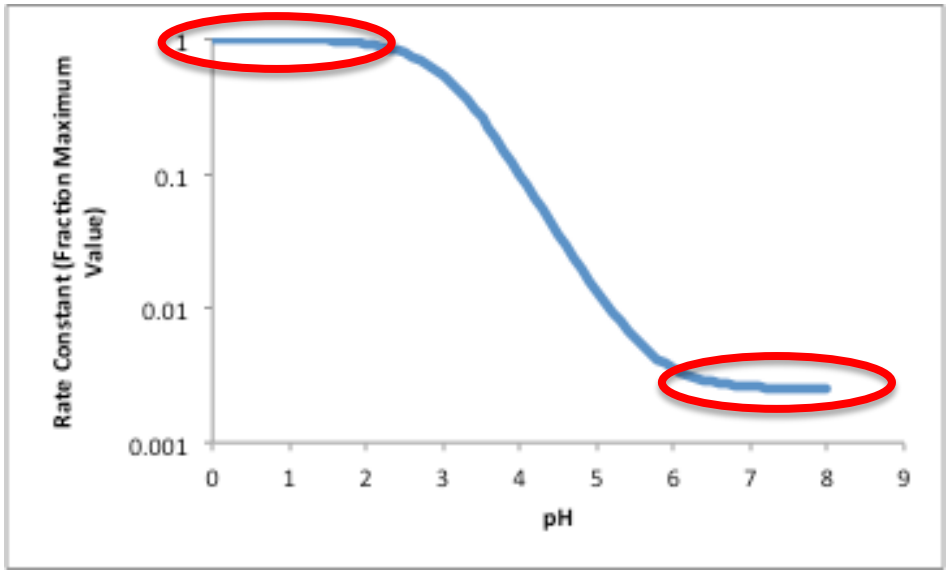
- We are in agreement that modeling of CrVI reduction in gastric content is important for the risk assessment
- Revised model for describing the reduction of CrVI in gastric contents based on the same *ex vivo* data used in Proctor et al. (2012) and Kirman et al. (2013).
- The complexity of the model is increased by:
  - *introducing multiple reducing agent pools (3 increased from 1 for rodents); and*
  - *introducing a chemistry basis for nonlinearity in the pH dependence by accounting for pH-dependent changes based on the form of chromium present.*
- Benefits
  - *The revised model provides improved fit to the ex vivo data.*
  - *Provides a quasi-chemistry basis for nonlinear relationship between reduction & pH*

Review of Schlosser and Sasso (2014) (cont'd)

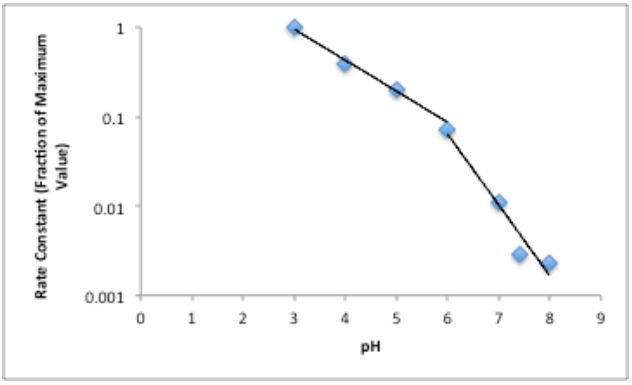
- However, model as parameterized creates an "apparent" species difference
  - Rodents - 3 reducing agent pools
  - Humans - 1 reducing agent pool
  - *Likely an artifact of how these data were collected rather than a true species difference, which could impact the risk assessment.*
    - Human samples were used to characterize pH dependency at relatively low concentrations
    - Rodent samples were used to characterize CrVI concentration dependency, including high concentrations associated with NTP bioassay
  - *To some extent the difference may also reflect different states: fed (rodent) and fasted (human)*
  - *To remedy this apparent species difference, additional ex vivo data have been collected using human samples*
    - Data support the presence of at least 2 reducing agent pools in both fed and samples.
- In addition, there are two behaviors of the pH-dependent model of Schlosser and Sasso (2014) that appear inconsistent with published data.

Reduction rate plateaus are not observed with specific reducing agents

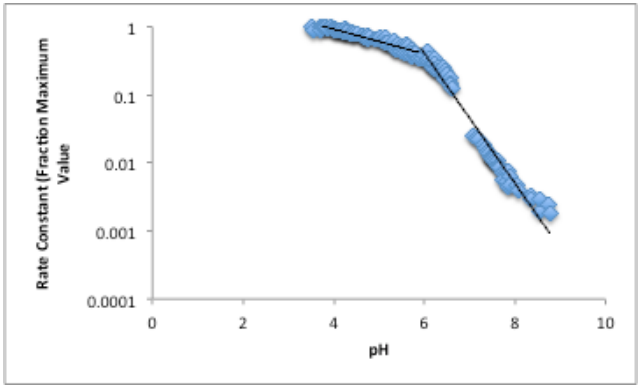
A) Proposed pH Dependence of Schlosser and Sasso (2014)



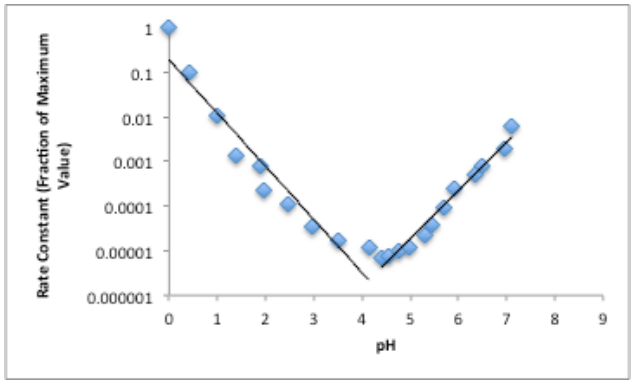
B) Glutathione (Wiegand et al., 1984)



C) Ascorbate (Dixon et al., 1984)



D) Iron (Buerge and Hug., 1997)



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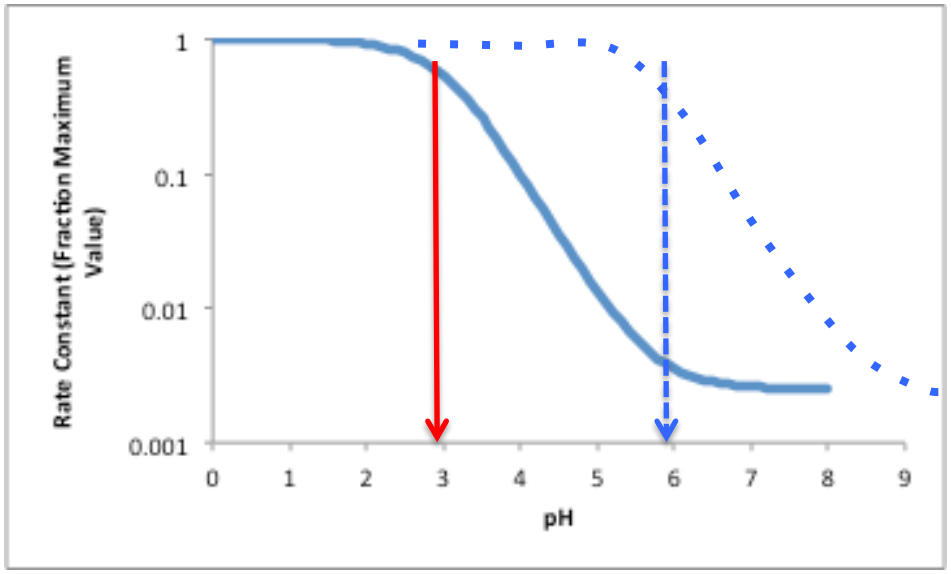
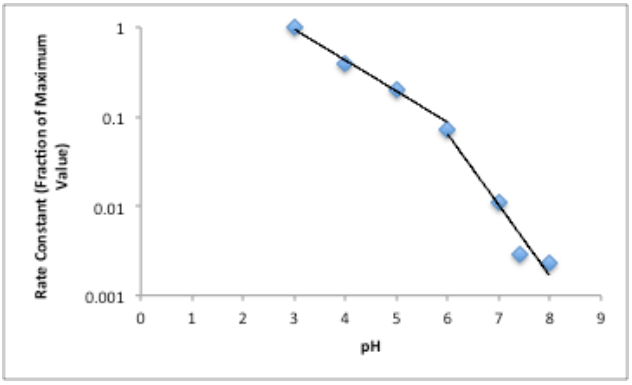
The pH where the inflection point occurs is too low

A) Proposed pH Dependence of Schlosser and Sasso (2014)



Inflection point (downturn in the curve) in the reduction rate is predicted above a pH of approximately 3. This inflection point is directly dependent upon the value adopted for pKa, which as indicated above was reduced from 5.9 to 3.0 (based on optimization to a small number of samples)

B) Glutathione (Wiegand et al., 1984)



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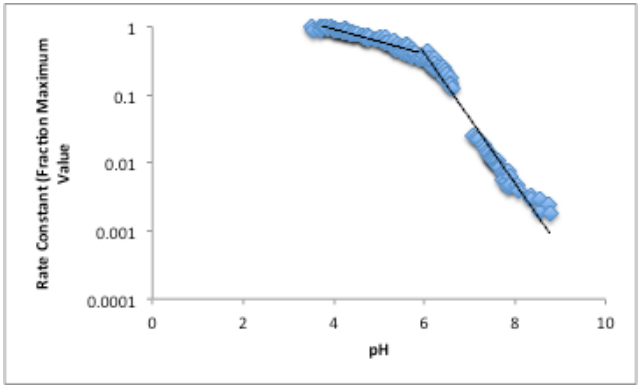

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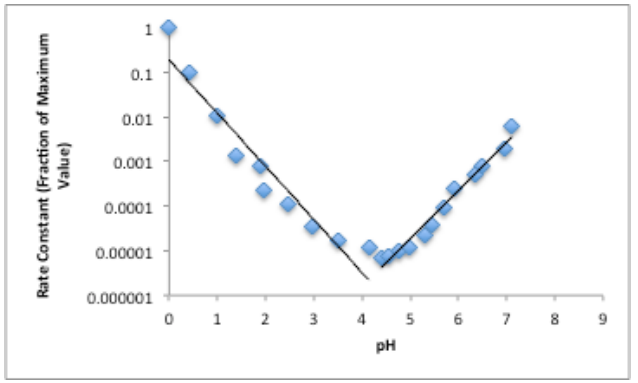

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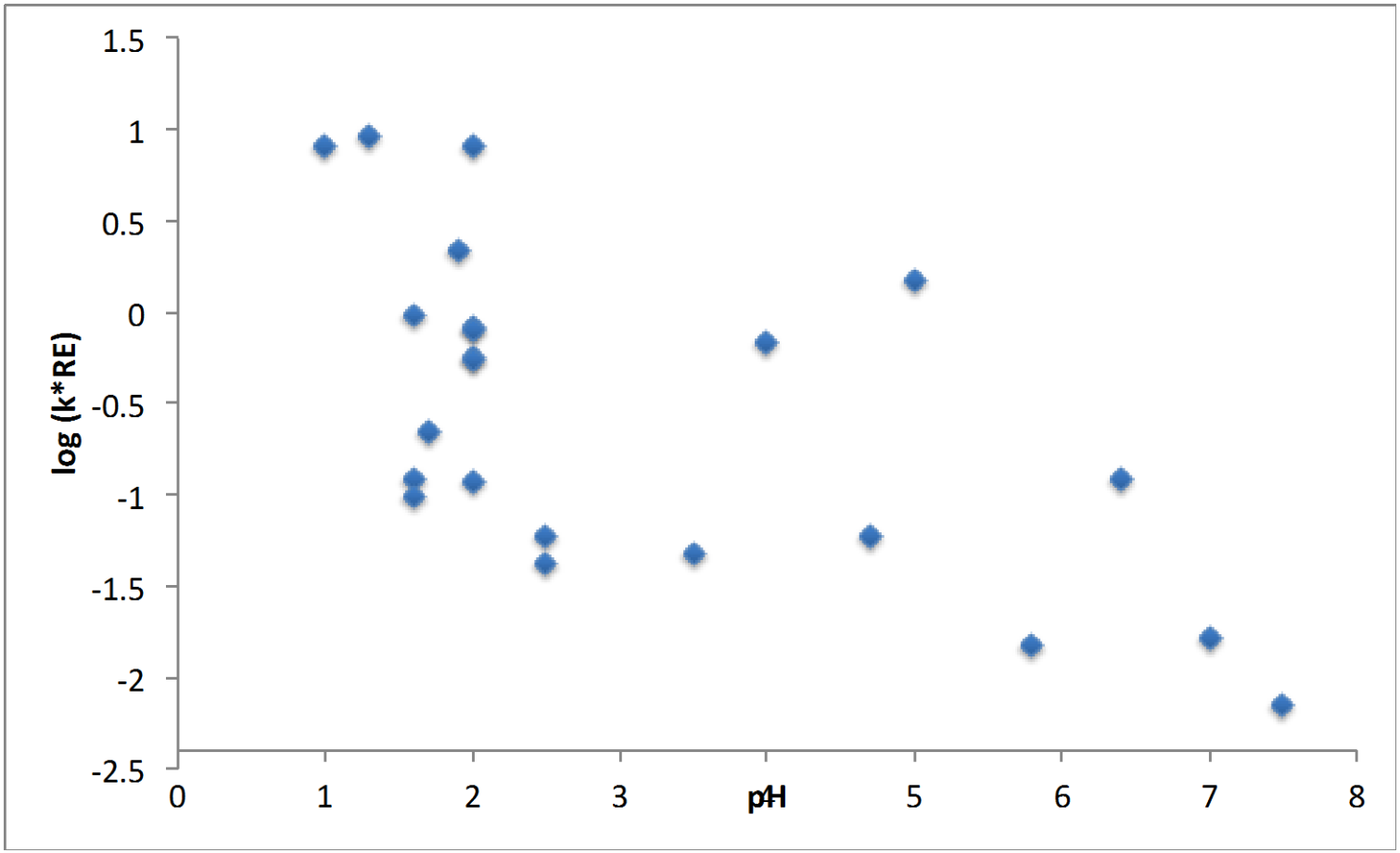
pH Dependence of CrVI reduction in Human Gastric Samples (Kirman et al., 2013; combined new data)



No clear evidence of plateaus or inflection points

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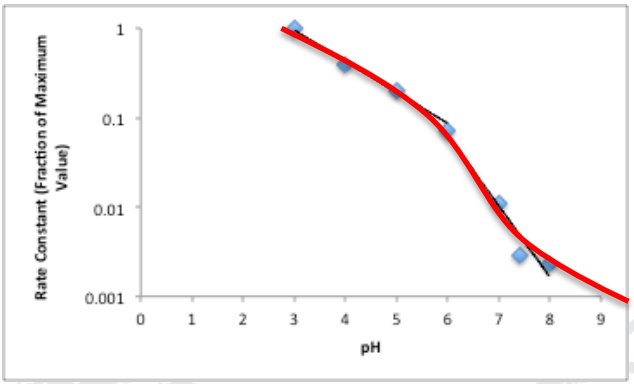
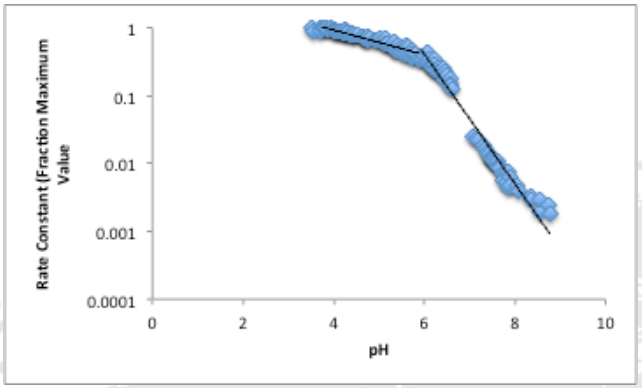
Why aren't we seeing evidence of plateaus or inflection points in the ex vivo data using gastric sample data?

- Absence of plateaus
  - *Redox potentials of CrVI and reducing agents are pH dependent*
  - *Potential direct role for hydrogen ions in reduction reaction (Connett and Wetterhahn, 1983)*
- Absence of inflection points
  - *Large individual variation may mask subtle inflection points*
  - *The drop-off in activity for some reducing agents (sulfhydryls, ascorbate) at higher pH may be masked by increase in activity for iron*

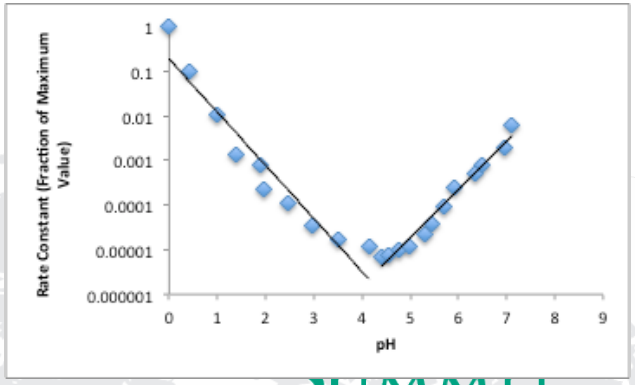
Glutathione



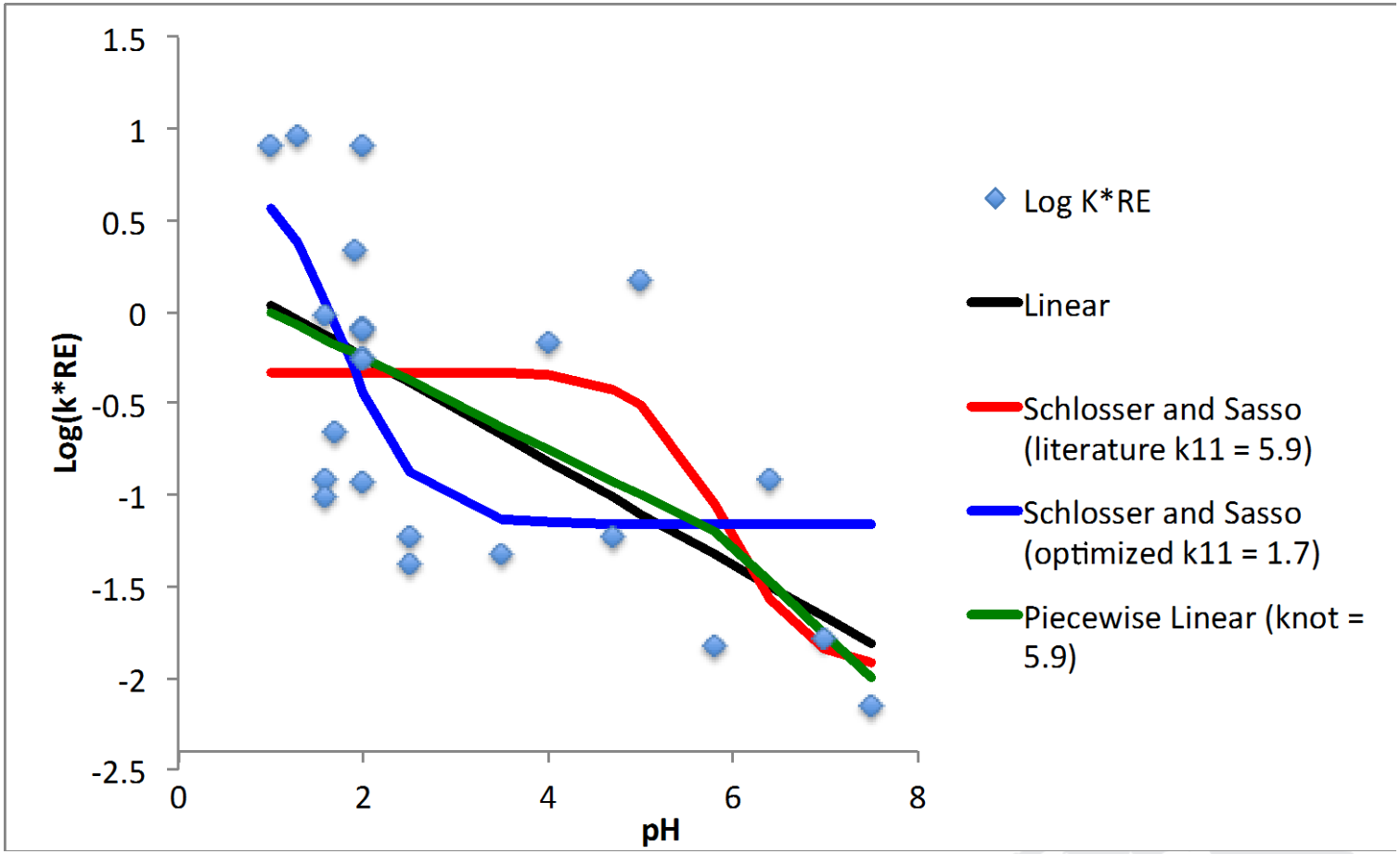
Ascorbate



Iron



Preliminary Comparison of pH-Dependent Model Forms



Preliminary results indicate linear form yields the lowest AIC (best fit to the data)

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Recommendations

- Incorporate the new ex vivo reduction data into the model
- Include multiple pools for both rodents and humans (i.e., use same qualitative structure, but allow for quantitative differences)
- Reconsider pH dependence of the model

Use of Nonphysiologic Exposure Route Data

- Nonphysiologic exposure studies (iv, ip, intratracheal) are used in PBPK model development (USEPA 2014, Table 3-1)
- Data from these studies need to be interpreted with caution:
  - *Differences in Systemic Delivery of CrVI*
    - Absorption is low, with reduction occurring at the portal of entry
      - » Majority of the Cr reaching systemic tissues is expected to be as CrIII.
    - By bypassing processes at the portal of entry, high concentrations of CrVI are produced in systemic tissues
      - » Different toxicokinetic behaviors that may not be predictive of oral and inhalation exposures.
      - » Biliary excretion conclusions appears to be strongly impacted by route of administration
  - *Differences in Exposure Timing*
    - Exposures to CrVI in drinking water or in air occur over an extended period of time.
    - In contrast, a bolus dose of CrVI to systemic compartments over a very short time period.
    - Potential to exceed the capacity of the compartment to reduce CrVI or for CrIII binding to transport proteins (transferrin)
      - » Both of which are expected to produce different toxicokinetic behaviors that may not be predictive of oral and inhalation exposures.

Use of Nonphysiologic Exposure Route Data

Studies that used multiple routes of exposure highlight TK behavior differences dependent upon route:

- Kargacin et al. (1993)
  - *Modest species differences for the distribution of Cr in blood following oral exposures to CrVI (rat>mouse).*
  - *Dramatic species differences following ip injection of CrVI (rat>>mouse).*
- Coogan et al. (1991)
  - *Time course data for Cr in red and white blood cells, found a much steeper decline in concentrations from day 1 to day 7 when CrVI was administered by oral gavage than by iv injection.*
- Sayato et al. (1980)
  - *Whole body retention of Cr decreased more slowly in rats following oral exposures to CrIII (half-life~92 days) compared to iv exposures to CrIII (half-life ~31 days).*

Use of Nonphysiologic Exposure Route Data

Based on these concerns, we ask the EPA to consider:

- Whether or not the oral and inhalation toxicokinetic studies are sufficient to stand on their own.
  - *For modeling oral exposures, we did not considered these to be needed*
- If data gaps are identified in the oral and inhalation studies, data from the injection and intra-tracheal instillation studies may be useful.
  - *Use of these studies may require characterization of the rate and capacity of CrVI reduction at the site of application,*
  - *May also require characterization of the rate and capacity of CrIII protein binding at the site of application*
- Data from the multiple routes of exposure studies may be used to validate any added complexity required.

Conclusions

- New Ex Vivo Data
  - *Multiple reduction pools (at least 2) are present in fed & fasted human gastric samples, with considerable inter-individual variation & no clear evidence of an inflection point in the pH relationship*
- Toxicokinetic Threshold
  - *Inclusion of multiple pools of reducing equivalents remains consistent with nonlinear toxicokinetics (potential inflection points as each pool is depleted)*
- Schlosser and Sasso (2014)
  - *Incorporate the new ex vivo reduction data into the model*
  - *Include multiple pools for both rodents and humans (i.e., use same qualitative structure, but allow for quantitative differences)*
  - *Reconsider pH dependence of the model (plateaus, inflection points)*
- TK Data from Nonphysiologic Exposure Data
  - *Use cautiously to supplement oral/inhalation data (if data gaps)*