Science Question 5: Cr-DNA Adducts

Key Points:

- **No empirical data support that Cr-DNA adducts occur *in vivo;* only mutagenic in highly contrived *in vitro* systems (Wise and Wise 2012; Thompson et al. 2013)**
- **Nuclear bioavailability of Cr(VI) is limited due to extracellular reduction and cytoplasmic trapping**
- **At Cr(VI) doses sufficient to damage DNA in mammalian cells, Cr(VI) is cytotoxic**
- **Current data do not support a role for Cr-DNA adducts in the MOA at know tumor sites (ingestion and inhalation)**

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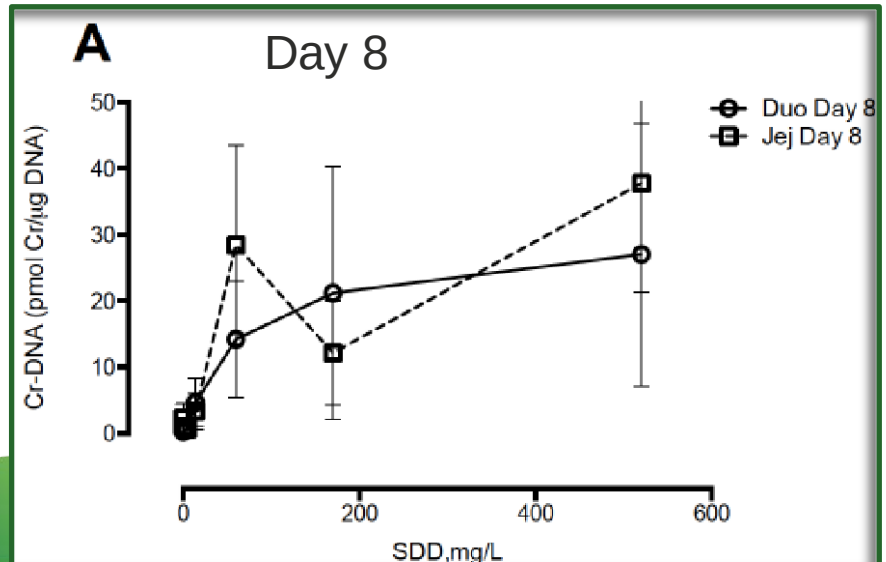
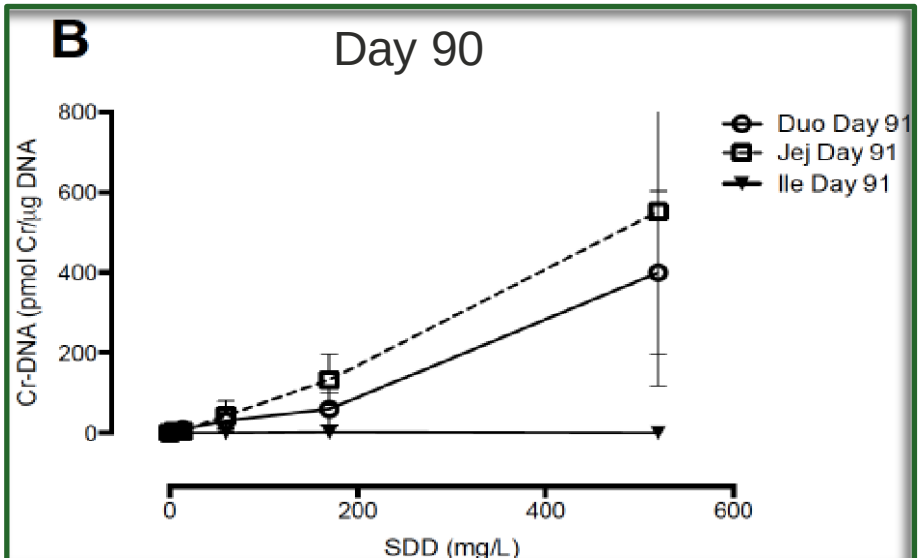
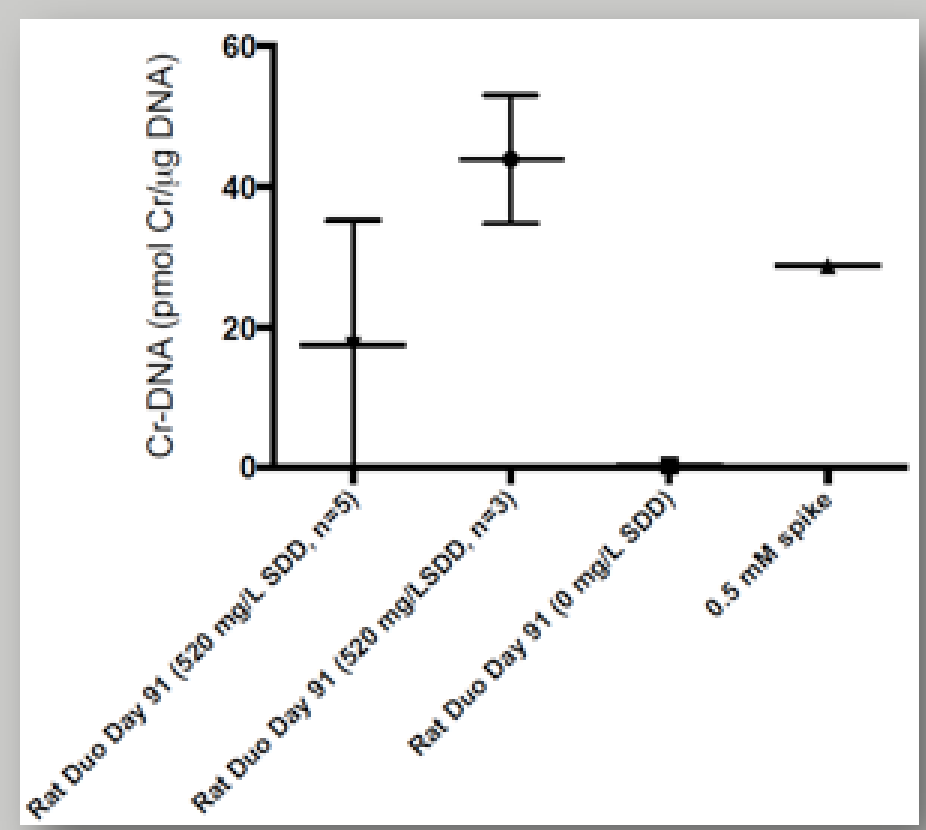
In Vivo Cr-DNA Binding (O'Brien et al. 2013-App B) [Funded by Cr(VI) Panel of ACC]

- **Collected Cr-DNA binding data *in vivo* in rat and mouse target tissues**
  - *Findings support results as biomarker of exposure*
  - *Current findings do not support a role for Cr-DNA adducts in the MOA for oral cavity and small intestine tumors*
- **Measured levels of Cr-DNA binding were not specific to responsive tissues**
  - *Cr-DNA binding was higher in the mouse jejunum than duodenum*
  - *Cr-DNA binding was increased in the mouse liver*
  - *Cr-DNA binding was elevated in the rat oral cavity at Day 8 than day 91 and levels were higher in the non-responsive mouse*





Cr-DNA Binding *In Vivo* Data Are Uncertain



Source: O'Brien et al. (2013) Appendix B

Quality Control Assessments Demonstrates Problems

- Cr-DNA binding occurs *ex vivo* during digestion/DNA extraction
- Cr-DNA binding was not reproducible
  1. Two analyses of Cr-binding in rat duodenum at 520 mg SDD/L result in significantly different results
  2. Cr-DNA binding in Cr(III)-spiked control rat intestine sample demonstrated high levels of Cr-bound to DNA

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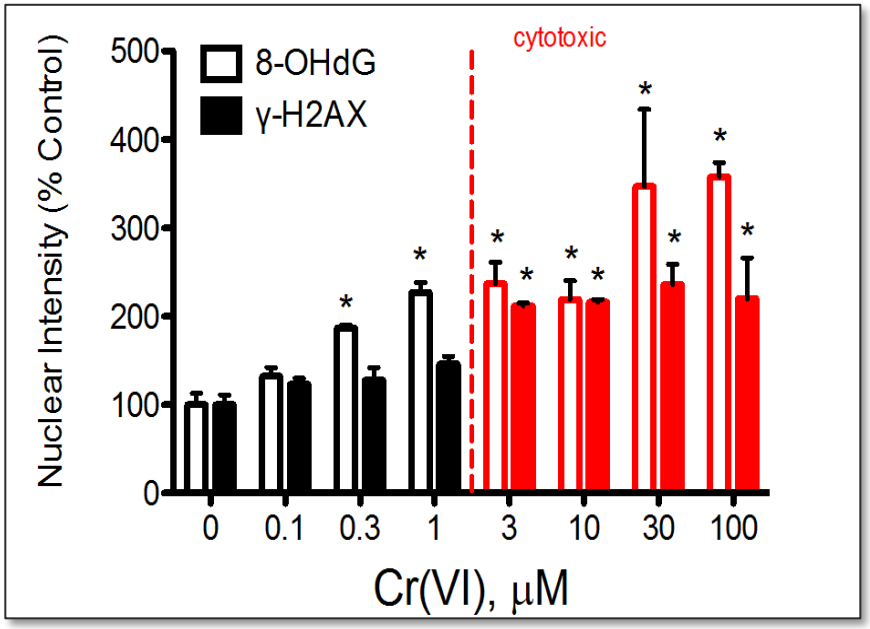
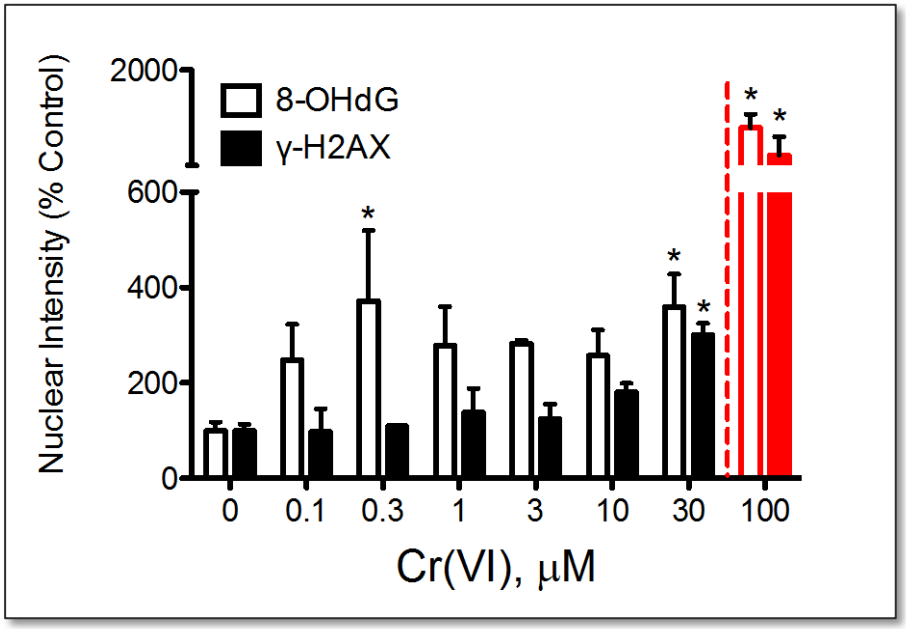
Cr(VI) Double Strand Breaks Occur at Cytotoxic Doses

Undifferentiated CACO-2



Differentiated CACO-2



1. Cr(VI)-induced double strand breaks (DSB) occur at cytotoxic concentrations—high dose effect
2. Oxidative DNA damage is a more sensitive effect than DSB

Source: Thompson et al. 2012 *Plos One*

Cytotoxic Doses

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No Evidence of Cr-DNA Adducts or Mutations in Cr(VI) MOA based on *In Vivo* Data

MOA for Intestinal MOA

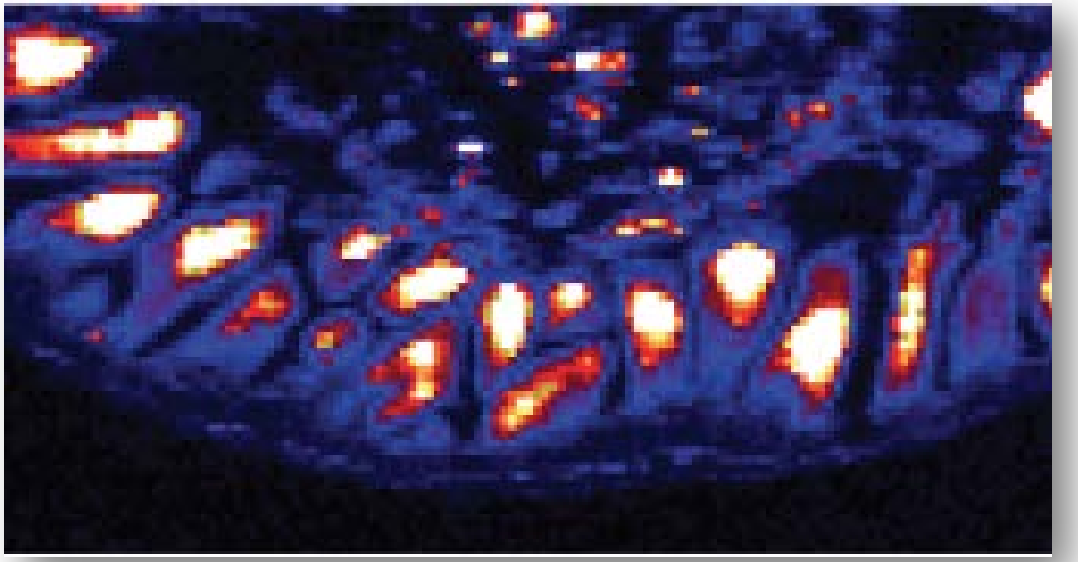
- Cr accumulated in mouse small intestinal villi, not crypts
- No increase in H2AX in intestinal crypts

(Thompson et al. accepted *Tox Sci*)

- No evidence of DNA damage or k-*ras* mutations in crypt (O'Brien et al. 2013)

MOA in Oral MOA

- No increase in mutations in rat oral cavity in Big Blue (In Review; as discussed for Question 7)



Role for Cr-DNA Adducts in Inhalation MOA is Not Supported by *In Vivo* Data

- **Cr-DNA adducts have not been reported *in vivo***
- ***In vitro* studies (e.g., Reynolds et al. 2004, 2007, and 2009) have shown that in human lung cells, Cr-DNA adduct is observed. However:**
  - Requires the use of plasmid vectors (Wise and Wise, 2012 referred to them as "experimentally contrived systems")
  - Supplementation with high levels of ascorbate (1.4 mM in Reynolds et al. 2007) is needed to from Cr-DNA adducts
  - Doses of Cr(VI) administered are cytotoxic. Cr-DNA adducts are observed when cell viability is low
- **Tumors in Cr(VI)-exposed workers show low P53 mutation frequency (Kondo et al. 1997)**
- **Animal data do not support mutagenic MOA in lung, oral cavity or intestine**
- **Overall, relevance of *in vitro* Cr-DNA adduct data is not supported based on the most recent MOA research data**