Discussion of the Derivation of an Oral Cancer Slope Factor for Hexavalent Chromium in Stern (2010)

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- This presentation provides a summary of the CSF derivation and its interpretation as published in

Stern AH (2010). A quantitative assessment of the carcinogenicity of hexavalent chromium by the oral route and its relevance to human exposure. Environ Res. 2010 Nov;110(8):798-807.

- To date, NJDEP has not incorporated this CSF into regulation
- NJDEP's current position is to await the EPA's revised IRIS assessment

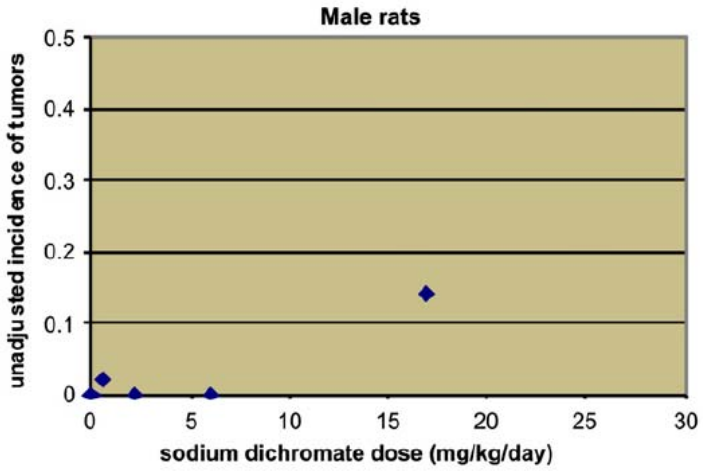
Summary of NTP 2008 Cr^{+6} Bioassay

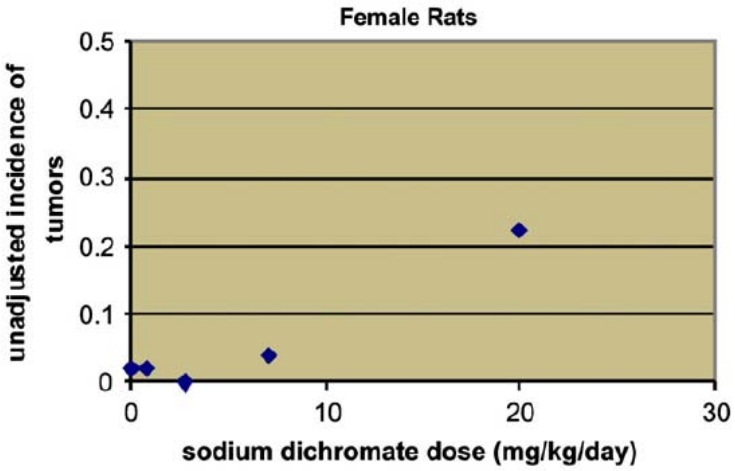
- M and F F344/N rats and B6C3F1 mice exposed to Cr^{+6} as sodium dichromate dihydrate in drinking water for 2 yrs.
- 50/sex/dose group
- 20% decrease in body wt. in F mice at high dose
  - Possible exceedance of maximally tolerated dose (MTD)
- All clinical signs normal in both species and sexes at all doses
- The only significant toxicity reported was neoplasms
  - Rats – oral mucosa and tongue
  - Mice - small intestine (duodenum, ileum and jejunum)

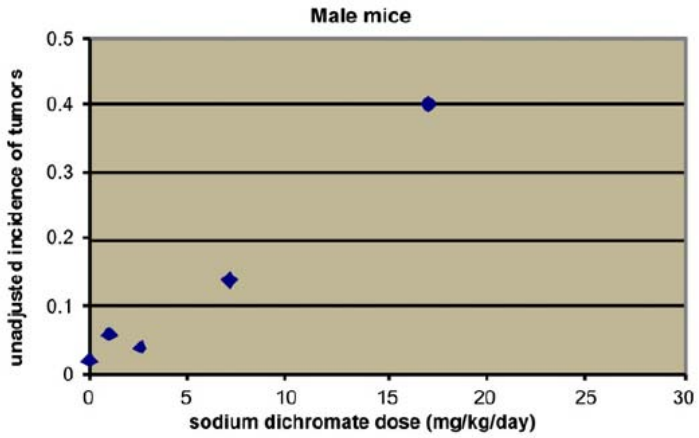
Dose-Response

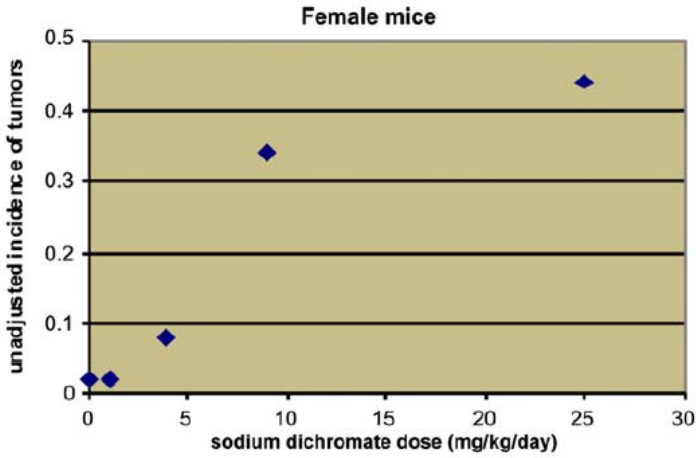
- Dose determined on the basis of water consumption and body wt.
  - Sodium dichromate is highly soluble and the Cr^{+6} was assumed to be 100% bioavailable

Unadjusted tumor incidence data for rats and mice from NTP 2008 study



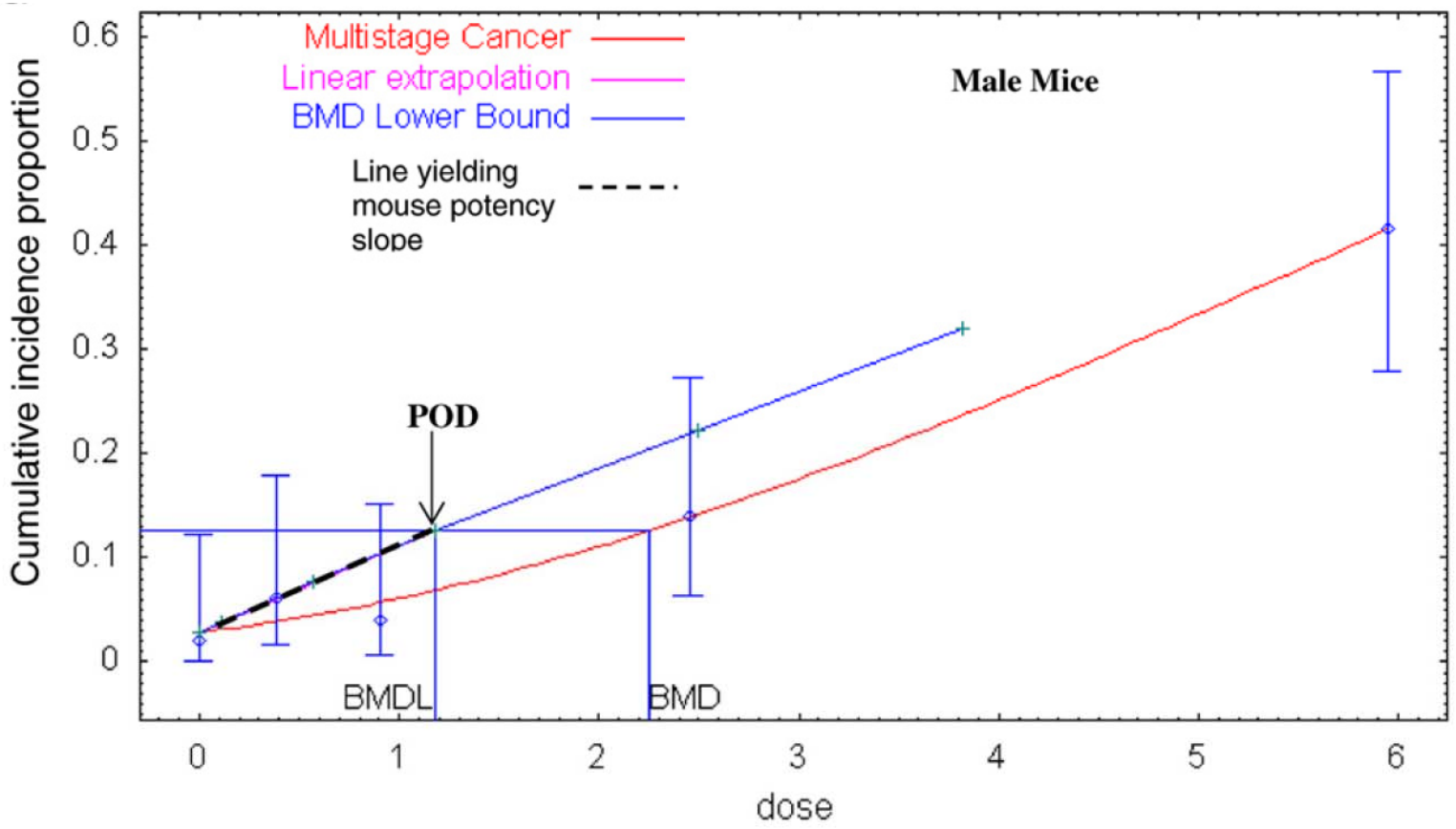






Estimation of Point of Departure (POD)

by benchmark dose modeling



Calculation of Cancer Slope Factor (CSF)

- M and F mouse data modeld
  - F mice poorly fit by benchmark dose suite of dose-response models
- Based on the POD
  - Lower 95% CI on the dose corresponding to the 10% tumor response in the best fit dose-response model
- Following EPA guidance, the mouse CSF is calculated as the slope of the line from the POD to the origin
- The human CSF is derived from the mouse CSF based on allometric interspecies pharmacokinetic scaling as $BW^{3/4}$
- Human CSF = 0.5 $(mg/kg/day)^{-1}$

Basis for linear extrapolation of the CSF

- EPA guidance (Guidelines for Carcinogen Risk Assessment – 2005) specifies linear extrapolation from the POD for mutagenic carcinogens or when there is insufficient evidence to support a non-linear extrapolation
- McCarroll et al. (2010)
  - An evaluation of the mode of action framework for mutagenic carcinogens case study II: chromium (VI). Environ Mol Mutagen. 2010 Mar;51(2):89-111. doi: 10.1002/em.20525.
- And Zhitkovich (2011)
  - Chromium in drinking water: sources, metabolism, and cancer risks.
  - Chem Res Toxicol. 2011 Oct 17;24(10):1617-29.
- Provide strong evidence for a mutagenic mode of action

- In addition, the oral cavity tumors in the rats in the NTP study occurred in the absence of necrosis or other tissue pathology
  - This is consistent with a mutagenic MOA
  - And not consistent with cell necrosis-repair MOA
- Occurrence of diffuse hyperplasia in the mouse duodenum, suggests that tissue damage and regeneration could have played a role in the formation of tumors in the mouse small intestine.
  - This is not evidence against a mutagenic MOA
  - Multiple overlapping MOAs could be operating

Summary of data quality in NTP study

- The database from the NTP study is judged to be of high quality.
- Well designed and well executed with no significant problems that raise questions about the validity of the results.
- Both the survival and the overall health of the animals were comparable to control animals at all doses with no clinical signs of toxicity.
- The decreased body wt. in F mice at highest dose may partly reflect moderate exceedance of MTD.
  - However, the cancer potency estimate was derived from the M mice and this did not influence the CSF estimate.

Cancer characterization under EPA (2005) guidelines

- ‘‘Likely to be carcinogenic to humans’’
  - ‘‘An agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans’’

Issues relating to Possible exceedance of a threshold for Cr^{+6} reduction

- It is known that the GI tract (stomach) has reserve capacity for the reduction of Cr^{+6} to Cr^{+3}
  - It has been argued that this capacity is sufficient for the reduction of relatively large doses of Cr^{+6} under extreme conditions of environmental contamination
  - Did tumors in NTP study exceed this reduction capacity (threshold)?
- Several lines of evidence argue against this

Pharmacokinetics of Cr^{+6} accumulation in peripheral tissue

- Cr^{+6} is absorbed more readily than Cr^{+3} from GI tract of rodents by factor of 1.8–60
- NTP conducted 2-yr oral dosing bioassay of chromium picolinate (Cr^{+3}) in parallel with sodium dichromate
- Concentration of total Cr measured in various tissues following 52 wks of dosing
- Total Cr in tissues was 2.1–38.6 times larger for mice ingesting Cr^{+6} compared to Cr^{+3}
  - Despite 2.8 times larger dose of Cr^{+3}
- Appears that Cr in these tissue must have been absorbed from GI tract as Cr^{+6}
  - i.e., without stoichiometric reduction.

Rate of accumulation of Cr in tissues as a function of dose

- Given slower rate of Cr^{+3} absorption, if threshold for reduction capacity were exceeded, we would expect to see an inflection point in the plot of tissue total Cr accumulation vs. dose
  - i.e., pre-threshold: slow absorption; post-threshold: more rapid absorption
  - Slope should increase after the excceedance of the reduction capacity
- Investigated using NTP data for various tissues at various time points
  - Mouse kidney, liver glandular and non-glandular stomach, plasma, erythrocyte, urine
- Statistical comparison of slope from lower portion of data set to slope of full data set.
  - Higher doses were obviously supra-linear and inconsistent with exceedance

- None of the tissues at any time point showed a statistically significant difference in slope that could be indicative of exceedance of a threshold for reduction
- This is consistent with non-exceedance of reduction capacity at any dose in the NTP study
  - Illustrated for blood and kidney (M mice) for 21 day exposure.

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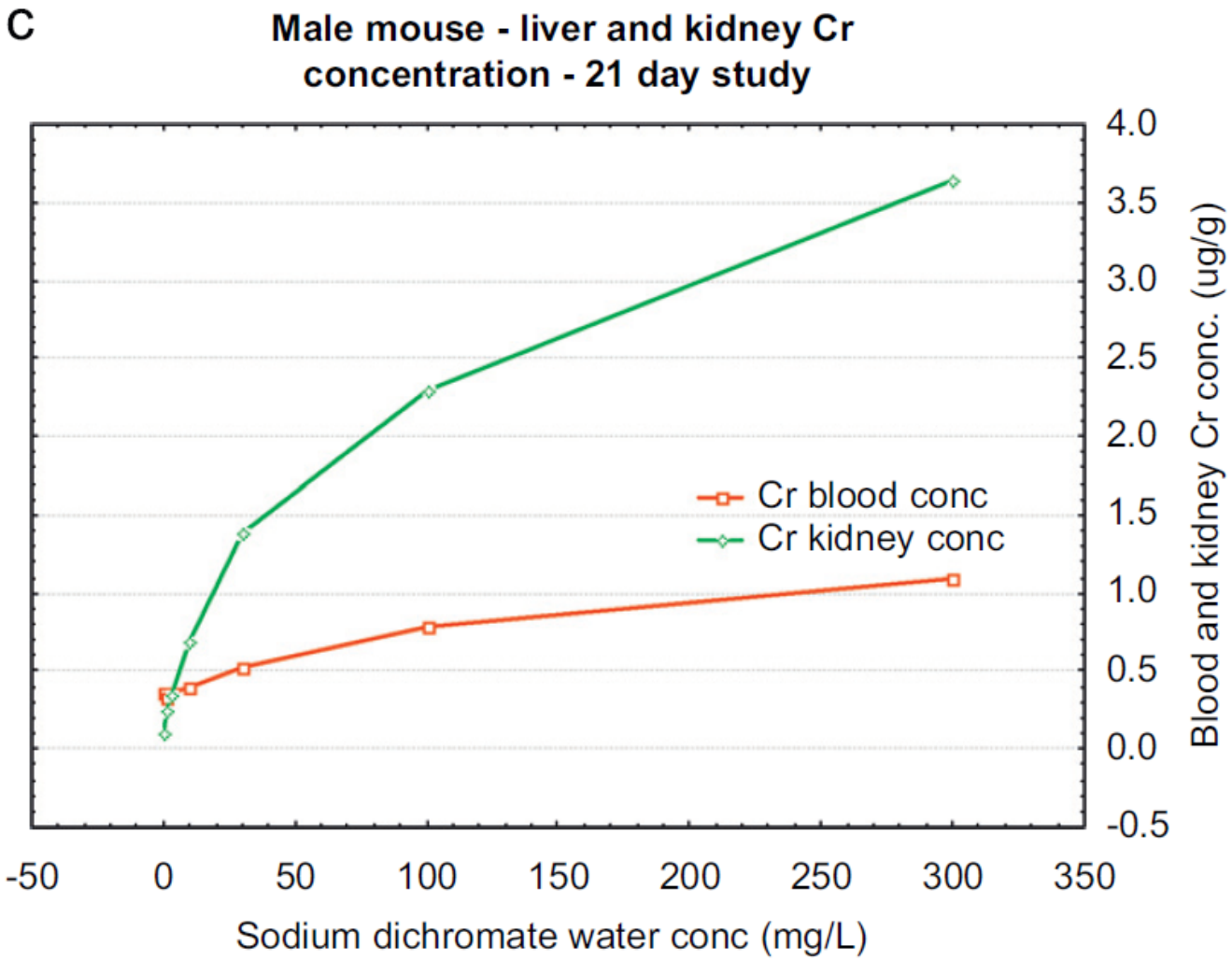
Male mouse - liver and kidney Cr concentration - 21 day study
Cr blood conc
Cr kidney conc
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Blood and kidney Cr conc. (ug/g)
-50
0
50
100
150
200
250
300
350
Sodium dichromate water conc (mg/L)

- This is additionally consistent with the observation that diffuse hyperplasia was observed in mouse duodenum at all sodium dichromate doses, but not in controls
  - i.e., diffuse hyperplasia was likely due to Cr^{+6} exposure and Cr^{+6} must have been present in the duodenum at all doses

Comparison of the Cr^{+6} intake rate and the reduction capacity of mouse gastric fluid

- No data on reduction capacity of mouse gastric fluid, but empirical data on reduction capacity of human gastric fluid
  - DeFlora et al. (1987, 1997)
- Estimated maximum Cr^{+6} reduction rate based on DeFlora et al. data is 10 mg Cr^{+6}/hr
- Processes governing reduction are likely under metabolic control
  - Production and secretion of the reducing chemicals, gastric mixing and gastric and small intestine emptying time
- Differences in meal-associated gastric reduction rate between mice and humans are, therefore, appropriately scaled on the basis of $(body\text{-}weight)^{3/4}$
  - As per EPA policy for interspecies extrapolation in dose-response

- Maximum Cr^{+6} intake rate for mice in NTP study is estimated from rate of peak period water consumption
- 12 hr dark period in NTP study
- Mouse maximum of Cr^{+6} intake rate (mg/hr) at each dose was compared to the estimated mouse Cr^{+6} reduction capacity (mg/hr)
- Cr^{+6} intake did not exceed estimated reduction capacity at any dose in M mice and only at highest dose in F mice
  - Nonetheless, intestinal tumors were observed in both sexes
- This analysis assumes that the stomach is a closed system and ignores the competing kinetics of gastric absorption and gastric emptying

Cr^{6+} water concentration (mg/L)	Mean peak period Cr^{6+} intake rate (assuming water consumption at 0.33 mL/h) (mg Cr^{6+}/h)	Estimated Cr^{6+} intake rate as a percentage of the estimated mouse gastric reduction capacity in mg Cr^{6+}/h
Male mice		
0	0	–
5	0.0017	3.9%
10	0.0033	7.5%
30	0.0099	22.5%
90	0.030	68.3%
Female mice		
0	0	-
5	0.0017	3.7%
20	0.0066	14.3%
60	0.020	43.5%
180	0.059	128.3%

Evidence for Cr^{+6} absorption at very low levels in mice

- Davidson et al. (2004) conducted an unusual study exposing hairless mice to Cr^{+6} in drinking water at only 3% of the lowest concentration in the NTP study. Followed by exposure to UV light
- Significantly more skin tumors were produced with co-exposure to Cr^{+6} +UV light compared to UV light alone
- The interpretation of this study in terms of carcinogenicity is unclear, but it suggests that to produce this result, Cr^{+6} must have been gastrointestinally absorbed
  - Despite the low dose and the reduction capacity of the mouse stomach

Human gastric reduction capacity and exposure to Cr^{+6}

- Evidence strongly suggests that mouse Cr^{+6} reduction capacity was not exceeded at any dose in the NTP study
- This raises the question of whether similar considerations also apply to human environmental exposures to Cr^{+6}

Human absorption of low dose Cr^{+6} compared to Cr^{+3}

- Kerger et al. (1996) administered Cr^{+3} and/or Cr^{+6} to human subjects
  - themselves
  - 5 mg each to 4 subjects
- Cr^{+6} dose was only ~18% of the lowest dose in NTP study
- For Cr^{+3}, mean peak urine concentration was 8.9 mg/g creatinine and a total of 0.13% of dose was recovered in urine.
- For Cr^{+6}, mean peak urine concentration was 209 mg/g creatinine and a total 6.9% of dose was recovered in urine
- It is clear that some Cr^{+6} escaped reduction and entered portal venous blood
  - Despite the relatively low dose of Cr^{+6} compared to the mouse doses in NTP

- The most direct explanation for this is that absorption from the human gastrointestinal tract is so rapid that it is able to compete effectively with reduction in the stomach (O'Flaherty et al., 2001).
- Kerger etal. (1996) alternatively proposedthat Cr^{+6} is reduced in the stomach to an organic complex of Cr^{+3} that is particularly absorbable
  - However as O'Flaherty et al. (2001) observed, this explanation is unlikely because ''no known complexes of Cr^{+3} are absorbed to the extent that Cr^{+6} is''
  - Further, Donaldson and Barreras (1966), showed that, in contrast to unreduced Cr^{+6}, gastrointestinally reduced Cr^{+6} was not absorbed through the (ex-vivo) small intestine

Conclusions

- Cr+6 is carcinogenic in rodents by the oral route of exposure
- There is strong evidence that Cr+6 has a mutagenic potential and it is reasonable to assume that rodent tumors in the NTP study occurred through a mutagenic MOA
  - Even if non-mutagenic processes were also present
- Cr+6 is appropriately characterized under EPA Carcinogen Guidelines as “Likely to be Carcinogenic to Humans”
- Multiple lines of evidence strongly suggest that the gastric reduction capacity for Cr+6 was not exceeded in the NTP study

- Observation in human subjects indicates that Cr+6 orall. administered at a concentration 18% of the lowest dose in the NTP study was systemically absorbed without reduction.
- It is likely that Cr+6 absorption reflects a dynamic competition between reduction and absorption/gastric emptying.
  - The latter process are relatively rapid compared to reduction
  - This probably reflects: gastric availability of the Cr+6, kinetics of absorption; kinetics of stomach emptying; and kinetics of the reduction reaction *per se*
- In the context of the EPA IRIS process, the CSF derived from the NTP data is well supported and of high quality.