Mode of Action Research for Cr(VI)-Induced Tumors: Overview & Highlight of Select Publications

Chad Thompson, PhD

Daniele Wikoff, PhD

October 8, 2020

ToxStrategies

Presentation Outline

- Notable updates and studies since our 2016 meeting
- Overview of MOA Research – Summary of the Emerging Science
  - Purpose and scope
  - Consistency with accepted MOAs
  - Evidence for lack of genotoxicity
  - Evidence for pharmacokinetic nonlinearities
- Recent Decisions on Toxicity Values
- Remaining Issues – Systematic Review, IRIS Schedule

Updates Since August 2016 Meeting

- Two publications demonstrating lack of mutagenicity in the rodent duodenum following exposure to Cr(VI)
- Updated PBPK models for Cr(VI)
- Updated oral reference dose for Cr(VI)
- Recently accepted manuscript (discuss at the end)
- Four agencies have proposed oral toxicity values based on nonlinear/threshold risk assessment approaches (e.g. RfD, TDI)

Recent Regulatory Values for Cr(VI) Have Relied on MOA Research

Agency	RfD or TDI (mg/kg-day)	Drinking Water (ppb)	Data Used
Health Canada (2016)	0.0022	50 (same as before)	NTP (SI hyperplasia) PBPK Models (dose metric) MOA Research (threshold extrapolation)
TCEQ (2016)	0.003	≅ 100	NTP (SI hyperplasia) PK Data (dose metric) MOA Research (threshold extrapolation)
Food Safety Commission of Japan (2019)	0.001	30-60	NTP (SI hyperplasia) MOA Research (threshold extrapolation)
WHO (2019, Draft)	--	50 (same as before)	MOA Research (retain existing value)

ToxStrategies

Since 2016, at least four agencies have developed regulatory values for oral exposure to Cr(VI) using nonlinear risk assessment methods. These agencies have based their assessments on the weight of evidence that the tumors observed in the NTP (2008) bioassay were not the result of a mutagenic MOA. Health Canada and TCEQ used both MOA and pharmacokinetic information from our studies (Health Canada, 2016; TCEQ, 2016), whereas the Food Safety Commission of Japan and WHO mostly relied on MOA information from our studies (FSCJ, 2019). Health Canada, TCEQ, and the Food Safety Commission of Japan all based their assessments on diffuse epithelial hyperplasia in the mouse small intestine reported in the NTP (2008) cancer bioassay. The WHO concluded that an older value they derived did not require updating based on the new available science (WHO, 2019).

New Values are Similar to Existing IRIS Value (1998)

I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
None Reported **Rat, 1-year drinking water study** **MacKenzie et al., 1958**	NOAEL: 25 mg/L of chromium as K_2CrO_4 2.5 mg/kg-day (adj.) LOAEL: None	300	3	3E-3 mg/kg-day

I.A.5. Confidence in the Oral RfD

Study — Low
Database — Low
RfD — Low

II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

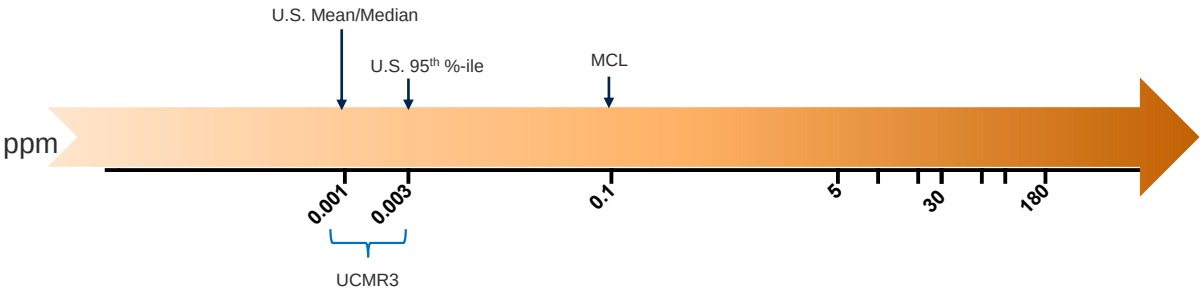
The oral carcinogenicity of Cr(VI) cannot be determined. No data were located in the available literature that suggested that Cr(VI) is carcinogenic by the oral route of exposure.

New values address cancer and confidence in RfD

5

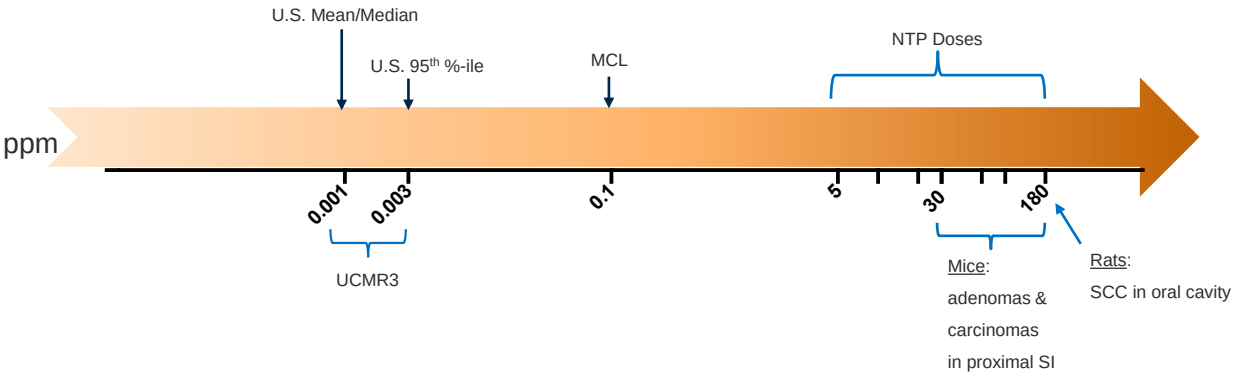
The existing IRIS RfD for Cr(VI) is based on the absence of toxicity in rats exposed to Cr(VI) in drinking water for 1 year (Mackenzie *et al.*, 1958). No evidence of carcinogenicity was observed at concentrations up to 25 ppm. IRIS characterized the confidence in the RfD was as low. As shown on the previous slide, several agencies have derived similar RfD values to the existing IRIS RfD value of 0.003 mg/kg-day. What is different is that the overall confidence in the study, database, and RfD calculation are improved based on the NTP 2-year bioassay, MOA information, pharmacokinetic modeling, and BMD modeling.

Cr(VI) MCL & US Water Levels



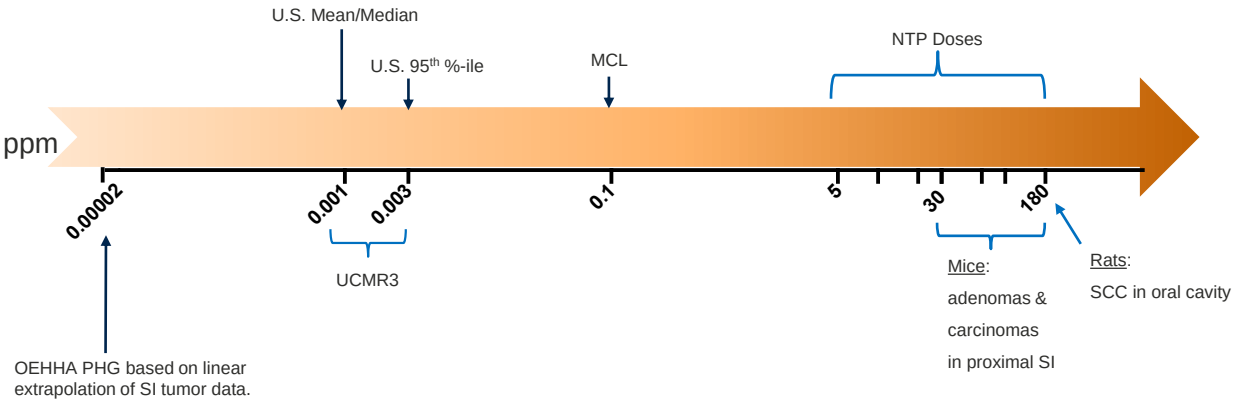
ToxStrategies

Cr(VI) MCL, US Water Levels, & NTP Doses



ToxStrategies

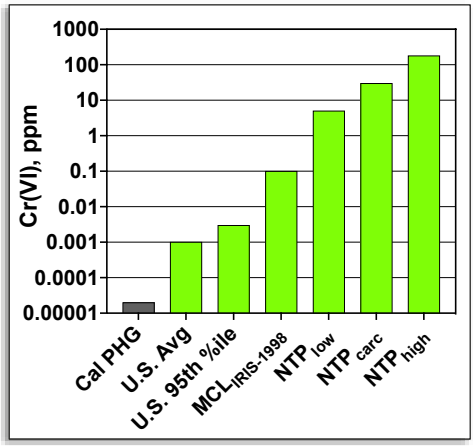
Cr(VI) MCL, US Water Levels, & NTP Doses



ToxStrategies

Is Linear Low Dose Extrapolation Scientifically Supported?

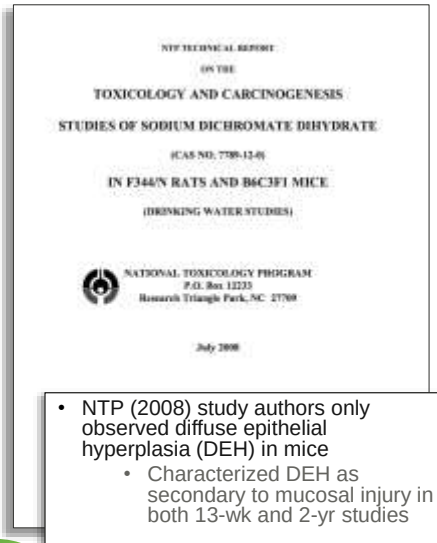
- Is there any evidence of a threshold MOA?
  - Evidence for cytotoxicity?
- Is there evidence for a mutagenic MOA?
  - Is there evidence of *in vivo* genotoxicity?
  - Target tissue genotoxicity?
- Is there evidence for pharmacokinetic nonlinearities?
  - Do environmental levels of Cr(VI) pose risk to the intestine?



ToxStrategies

Regulators at the New Jersey Department of Environmental Quality (NJDEP) derived an oral cancer slope factor based on small intestine tumors in male mice from the NTP bioassay (Stern, 2010). Default linear low dose extrapolation of the dose-response for intestinal tumors results in 1E-6 risk at water concentrations orders of magnitude below the average concentration of 0.001 ppm. In the 2010-2011 timeframe, organizations like EPA and OEHHA used or develop similar values. For example, the OEHHA public health goal (PHG) of 0.00002 ppm is based on intestinal tumors (OEHHA, 2011).

Early Suggestions of a Cytotoxicity/Cell Proliferation MOA



NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF SODIUM DICHROMATE DIHYDRATE
(CAS NO. 7789-12-0)
IN F344/N RATS AND B6C3F1 MICE
(DRINKING WATER STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

July 2008

- NTP (2008) study authors only observed diffuse epithelial hyperplasia (DEH) in mice
  - Characterized DEH as secondary to mucosal injury in both 13-wk and 2-yr studies

DEH in Duodenum (Females):

Cr(VI) ppm	Mice (13-wk)	Rats (13-wk)
0	0/10	0/10
22	7/10	0/10
44	8/10	0/10
88	10/10	0/10
175	10/10	0/10
350	10/10	0/10

2-year Study(Female Mice):

Cr(VI) ppm	DEH (Duo)	Tumor (SI)
0	0/50	1/50
5	16/50	1/50
20	35/50	4/50
60	31/50	17/50
180	42/50	22/50

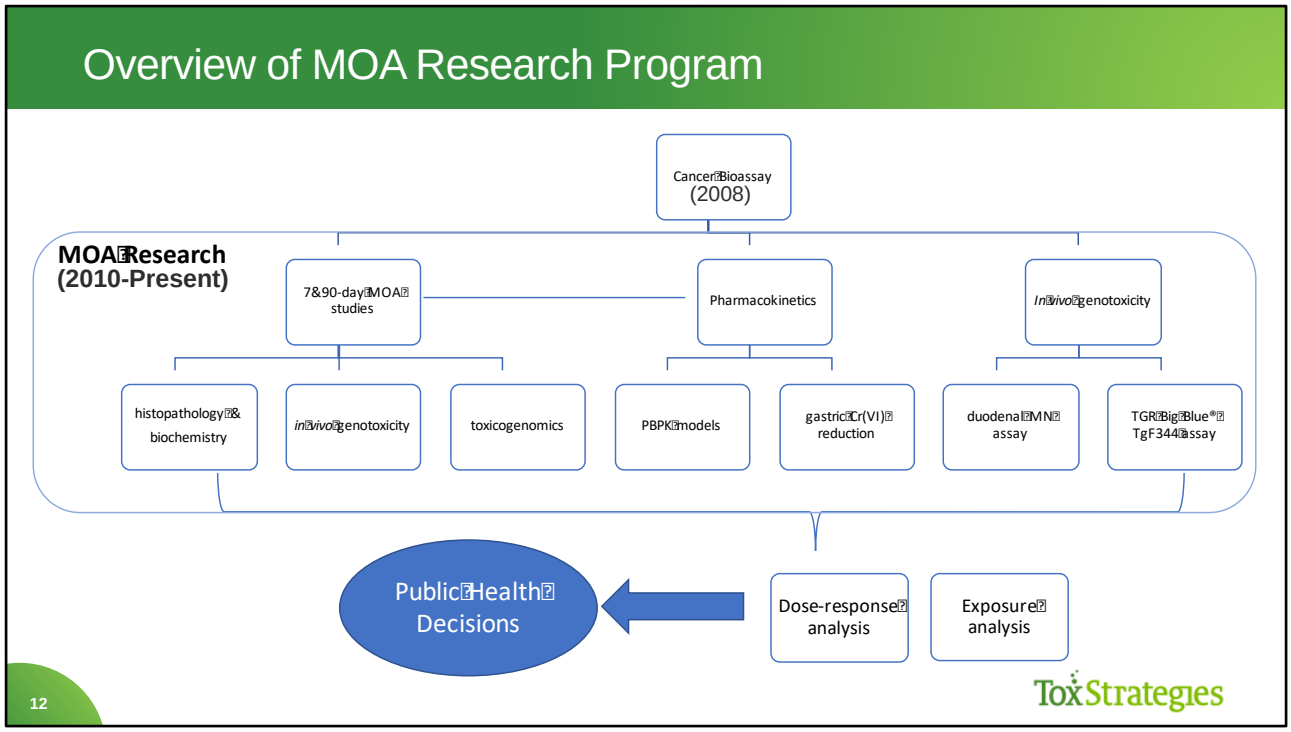
10

ToxStrategies

It is well documented that chemicals that induce cytotoxicity and cell proliferation in short term bioassay will likely cause cancer in the affected tissue in long term bioassays. In the NTP 13-week bioassay, NTP reported diffuse epithelial cell hyperplasia in the duodenum of mice but not rats (NTP, 2007). In the chronic bioassay, male and female mice exhibited dose-dependent increases in diffuse epithelial hyperplasia as well as duodenal tumors, whereas neither intestinal hyperplasia nor intestinal tumors were reported in rats (NTP, 2008). These data suggest a cytotoxicity/regenerative hyperplasia MOA.

MOA Research Overview

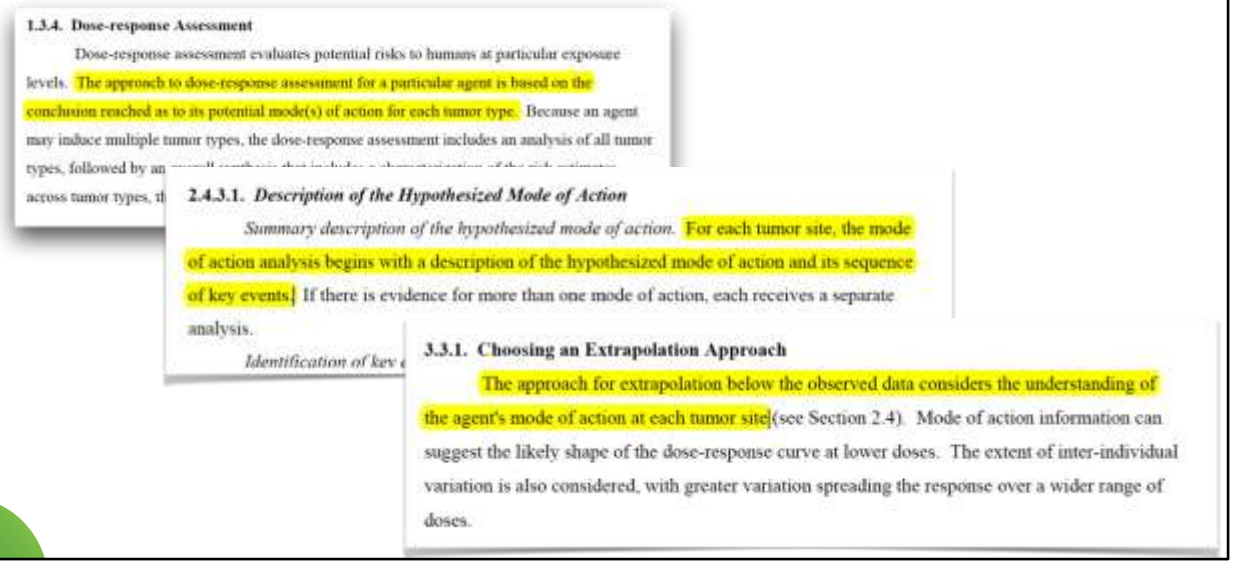
Tox Strategies



The MOA research was designed to inform the oral risk assessment of Cr(VI). Consistent with U.S. EPA Cancer Guidelines, it was designed to inform the MOA for the tumors associated with oral exposure to Cr(VI), i.e., oral tumors in rats and intestinal tumors in mice. The study began with a 90-day bioassay in female rats and mice (with a 7-day interim time point) that was conducted at Southern research, the same contract lab that conducted the NTP 2-year bioassay. Various analyses included histopathology, biochemistry, transcriptomics, pharmacokinetics, and assessment of in vivo genotoxicity in target tissues.

MOA is for the Tumor, Not the Carcinogen

U.S. EPA (2005) Guidelines for Carcinogen Risk Assessment:



1.3.4. Dose-response Assessment

Dose-response assessment evaluates potential risks to humans at particular exposure levels. The approach to dose-response assessment for a particular agent is based on the conclusion reached as to its potential mode(s) of action for each tumor type. Because an agent may induce multiple tumor types, the dose-response assessment includes an analysis of all tumor types, followed by an

across tumor types, t

2.4.3.1. *Description of the Hypothesized Mode of Action*

Summary description of the hypothesized mode of action. For each tumor site, the mode of action analysis begins with a description of the hypothesized mode of action and its sequence of key events. If there is evidence for more than one mode of action, each receives a separate analysis.

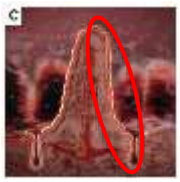
Identification of kev

3.3.1. Choosing an Extrapolation Approach

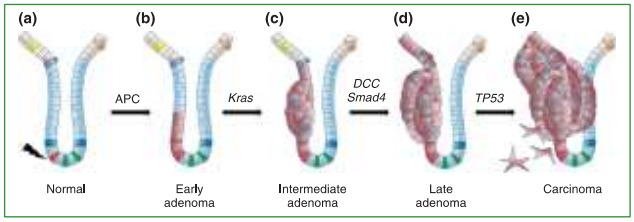
The approach for extrapolation below the observed data considers the understanding of the agent's mode of action at each tumor site (see Section 2.4). Mode of action information can suggest the likely shape of the dose-response curve at lower doses. The extent of inter-individual variation is also considered, with greater variation spreading the response over a wider range of doses.

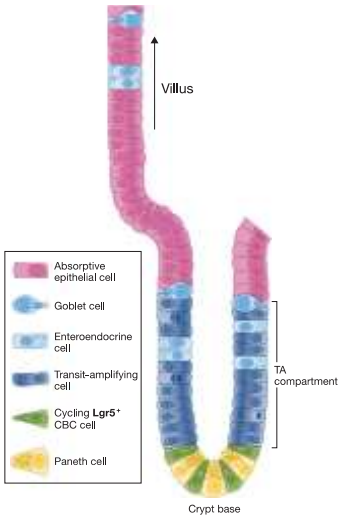
13

Small Intestine Structure and Carcinogenesis



Model of Intestinal Cancer Initiation & Progression



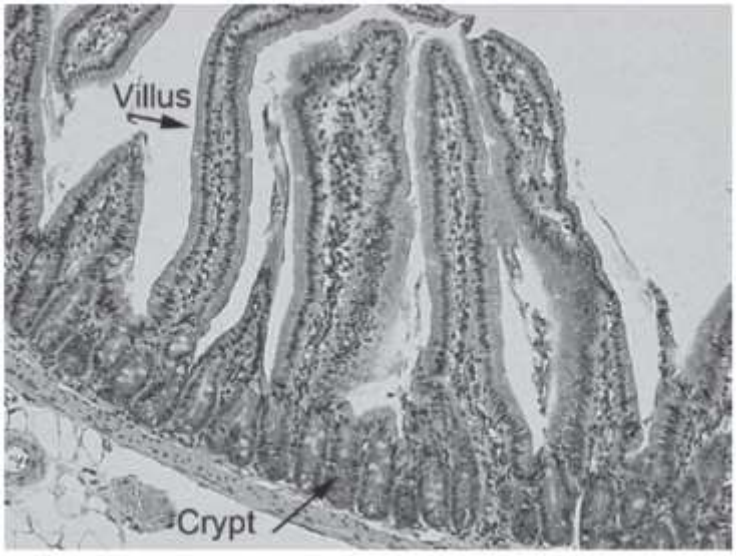


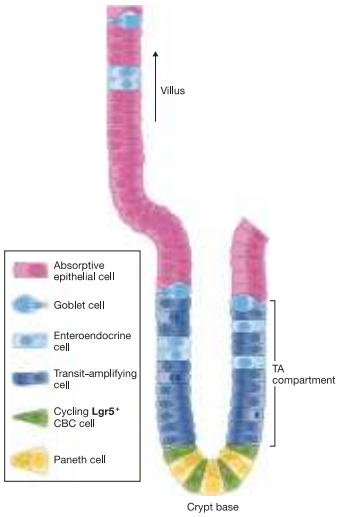
Sources: Schuijers & Clevers (2012) EMBO J. 31, 2685.
Rizk & Barker (2012) WIREs Syst Biol Med. 4, 475.

14

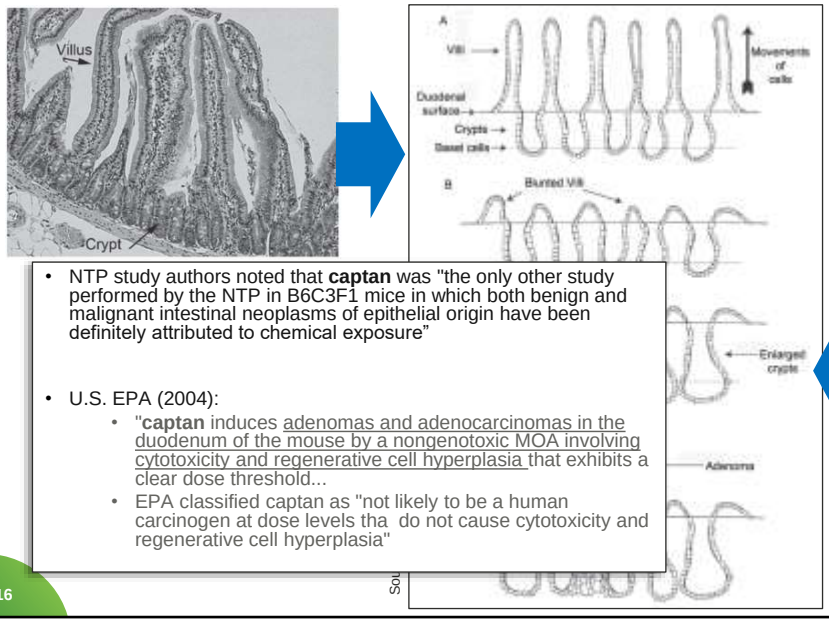
Because the intestinal tumors in mice occurred at lower exposure concentrations than oral tumors in rats, most of the research has been focused on the small intestine. The small intestine has a unique structure. It is generally believed that cancer arises from mutations in stem cells at the base of crypts, which are small invaginations in the intestinal surface. These stem cells give rise to daughter cells that move up through the crypt and out onto villi. Proliferating enterocytes become terminally differentiated upon reaching the villi. Toxicity in these villous enterocytes is not likely to result in the direct formation of cancerous cells.

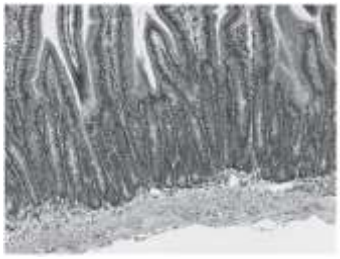
Small Intestine Histopathology





Published MOA for SI Tumors in Mice: Folpet/Captan





- NTP study authors noted that **captan** was "the only other study performed by the NTP in B6C3F1 mice in which both benign and malignant intestinal neoplasms of epithelial origin have been definitely attributed to chemical exposure"
- U.S. EPA (2004):
  - "**captan** induces adenomas and adenocarcinomas in the duodenum of the mouse by a nongenotoxic MOA involving cytotoxicity and regenerative cell hyperplasia that exhibits a clear dose threshold...
  - EPA classified captan as "not likely to be a human carcinogen at dose levels tha do not cause cytotoxicity and regenerative cell hyperplasia"

16

ToxStrategies

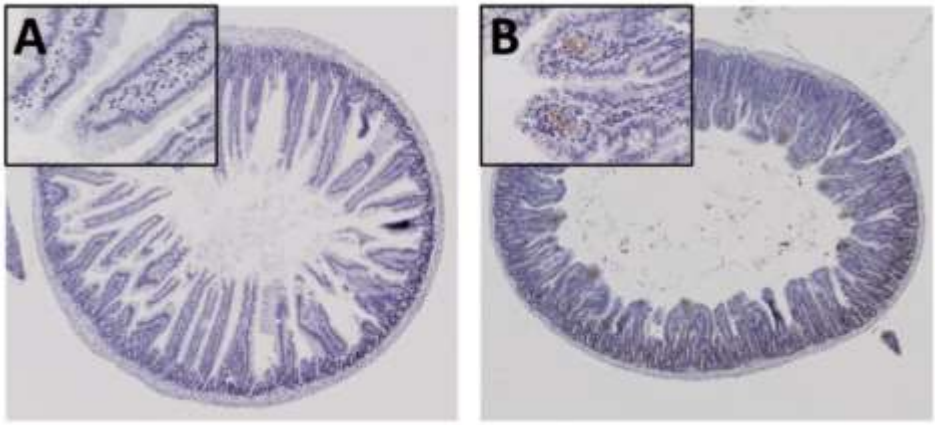
A mode of action for captan- and folpet-induced intestinal cancer was proposed over a decade ago (Cohen *et al.*, 2010). Toxicity to villous enterocytes leads to accelerated cell loss and thus a regenerative proliferative response in the intestinal crypts. If the cell loss is severe, the crypt is unable to regenerate the normal villus structure and thus results in villous blunting/atrophy. Under prolonged cytotoxic exposures, the increased stem cell proliferation results in an increased probability of DNA replication error, transformation, and tumor development. The MOA for captan has been accepted by regulators such as EPA. Notably, the NTP Cr(VI) study authors specifically mentioned captan as the only other long term NTP study to induce similar tumors in B6C3F1 mice (NTP, 2008; Stout *et al.*, 2009).

Histopathology in Mice Following Cr(VI) Exposure: Villus Blunting

90 Days Exposure:

Tap Water

180 ppm Cr(VI)



17

ToxStrategies

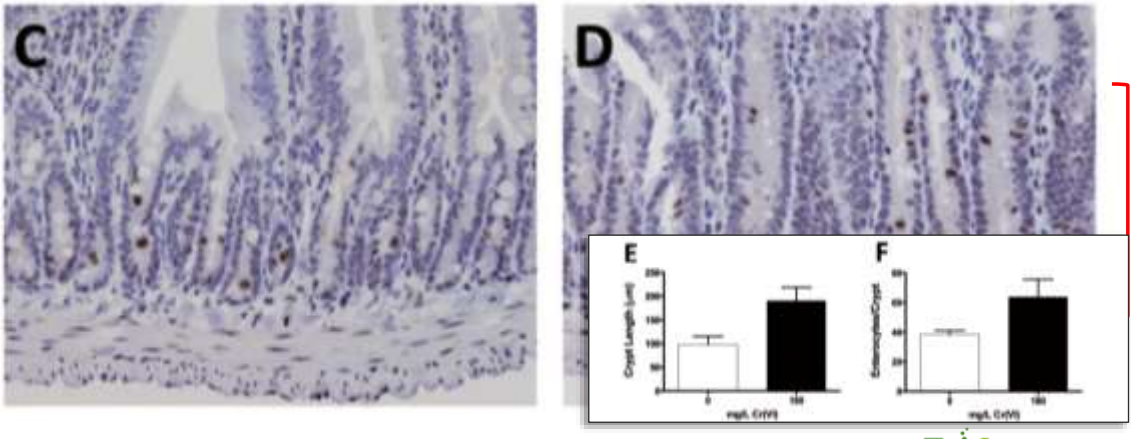
These slides show the normal cross section through the mouse duodenum on the left, and villous blunting (note wider luminal space) in the duodenum of mice exposed to 180 ppm Cr(VI) for 90 days (right) (Thompson *et al.*, 2015a). This phenotype is consistent with villous changes induced by captan and folpet.

Histopathology in Mice Following Cr(VI) Exposure: Crypt Expansion

90 Days Exposure:

Tap Water

180 ppm Cr(VI)



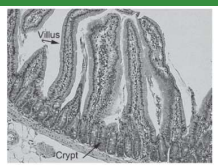
18

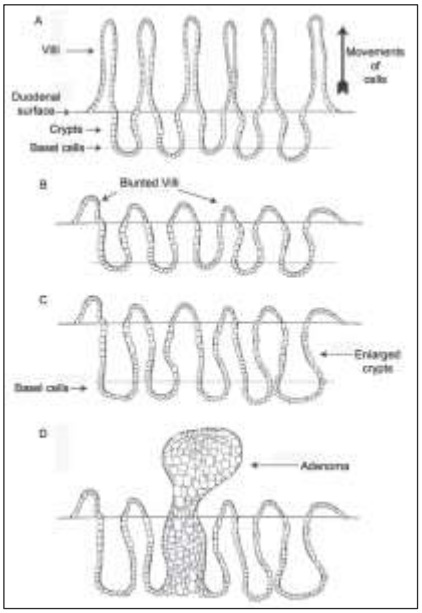
ToxStrategies

These slides (also adapted from Thompson et al., 2015a), show the normal height of the crypt compartment in control mice (left), and crypt elongation due to increased cell proliferation in the duodenum of mice exposed to 180 ppm Cr(VI) for 90 days (right). This phenotype is consistent with crypt changes induced by captan and folpet. Changes to the crypt height (or depth) have been quantified in terms of length and cell number (inset).

Cr(VI) Has Similar Phenotype to Folpet and Captan

Folpet:



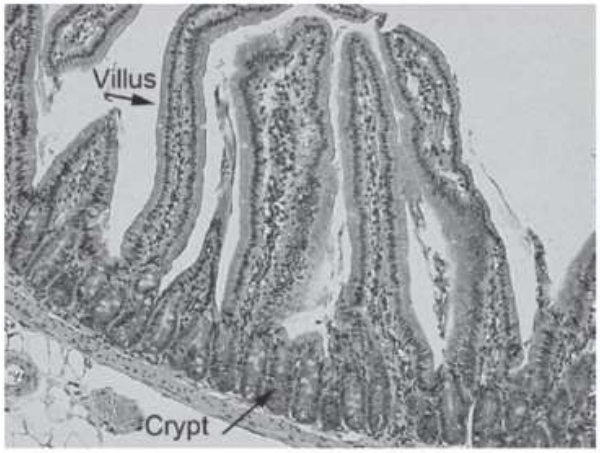


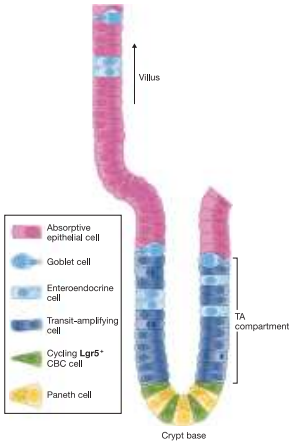
Source: Cohen et al. (2010) Crit Rev Toxicol 40: 531.

:Cr(VI)

ToxStrategies

Critical Question: Where Is Chromium Localized in the Duodenum?

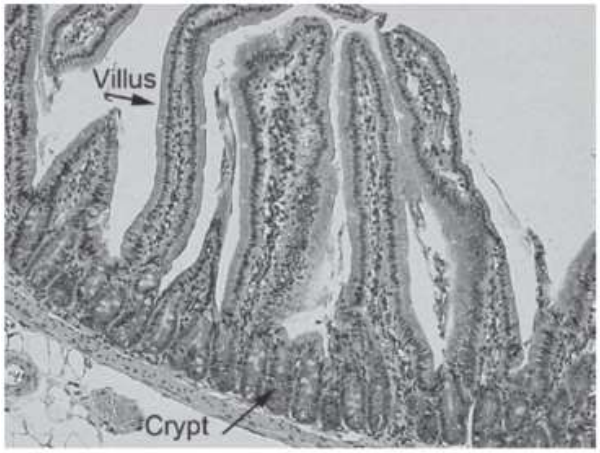


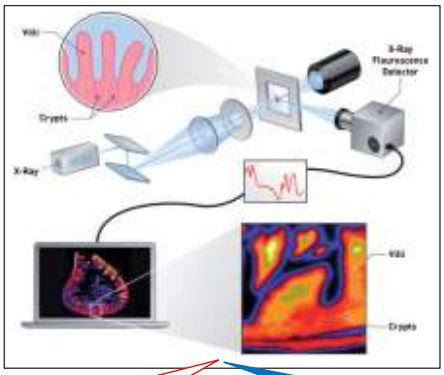


20

Given the structure of the duodenum, it is important to understand where chromium is specifically localized in the intestinal mucosa. Detection in the crypts might support direct acting mutagenic MOA. In collaboration with the U.S. Army Corps of Engineers, unstained tissue sections were taken to the Brookhaven National Laboratory synchrotron where duodenal sections were exposed to x-ray beams to excite various elements (e.g., Cr) in fixed tissue to fluoresce and thereby allow visualization of location.

Critical Question: Where Is Chromium Localized in the Duodenum?





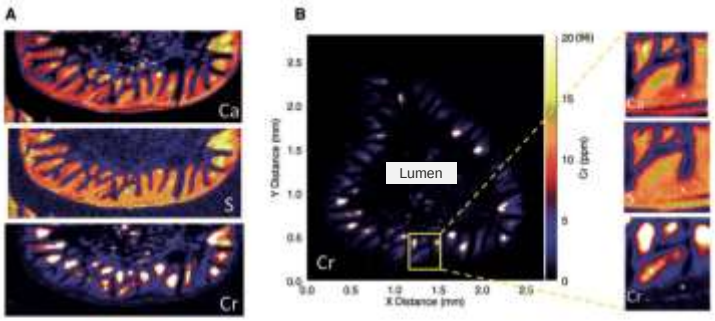
U.S. Army Corps of Engineers

Brookhaven National Laboratory

21

Given the structure of the duodenum, it is important to understand where chromium is specifically localized in the intestinal mucosa. Detection in the crypts might support direct acting mutagenic MOA. In collaboration with the U.S. Army Corps of Engineers, unstained tissue sections were taken to the Brookhaven National Laboratory synchrotron where duodenal sections were exposed to x-ray beams to excite various elements (e.g., Cr) in fixed tissue to fluoresce and thereby allow visualization of location.

Cr is Detected in the Villus Region of the Duodenal Mucosa

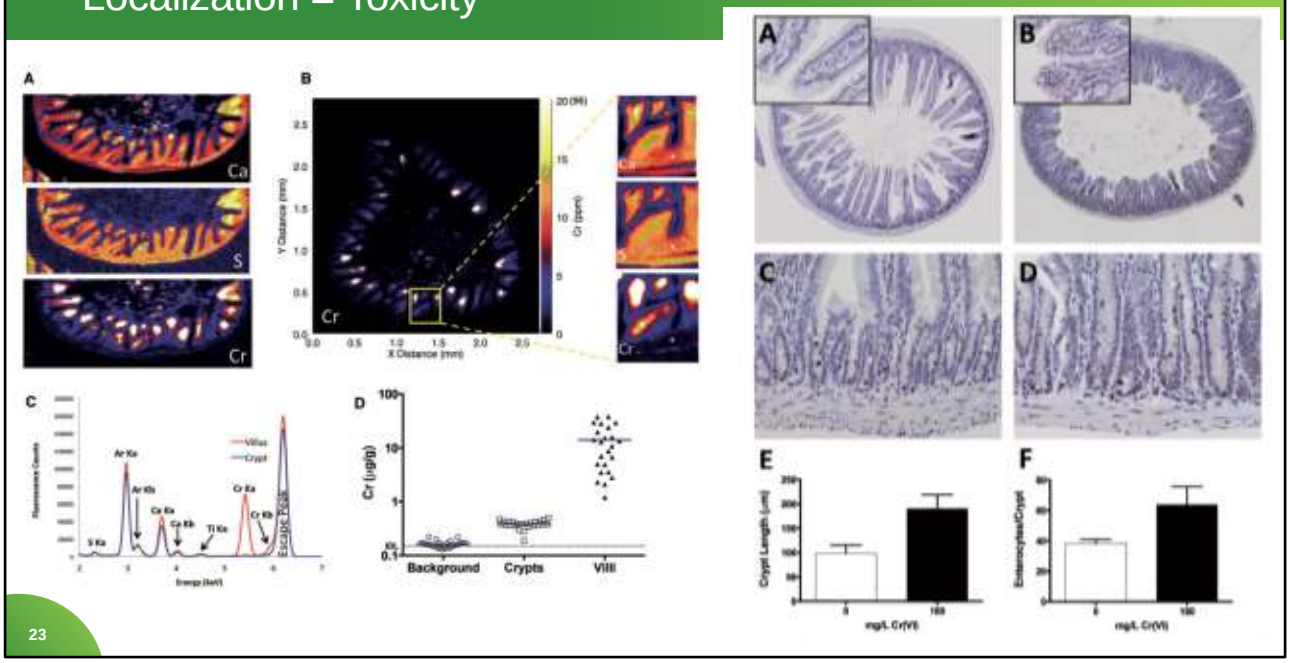


22

ToxStrategies

In this cross section of a mouse exposed to 180 ppm Cr(VI) for 90 days, XRF imaging reveals the presence of calcium (Ca) and sulfur (S) fluorescence throughout the crypts and villi (Thompson *et al.*, 2015a). In contrast, Cr(VI) is readily detected at villous tips and is nearly absent in the crypts. This indicates that toxicity most likely occurs in the villi, and not in the crypts or stem cells near the base of the crypt. Critically, this localization is consistent with the histopathology shown earlier indicating healthy elongating crypts, and blunting of the villi. These data also inform results from genotoxicity testing.

Localization = Toxicity



23

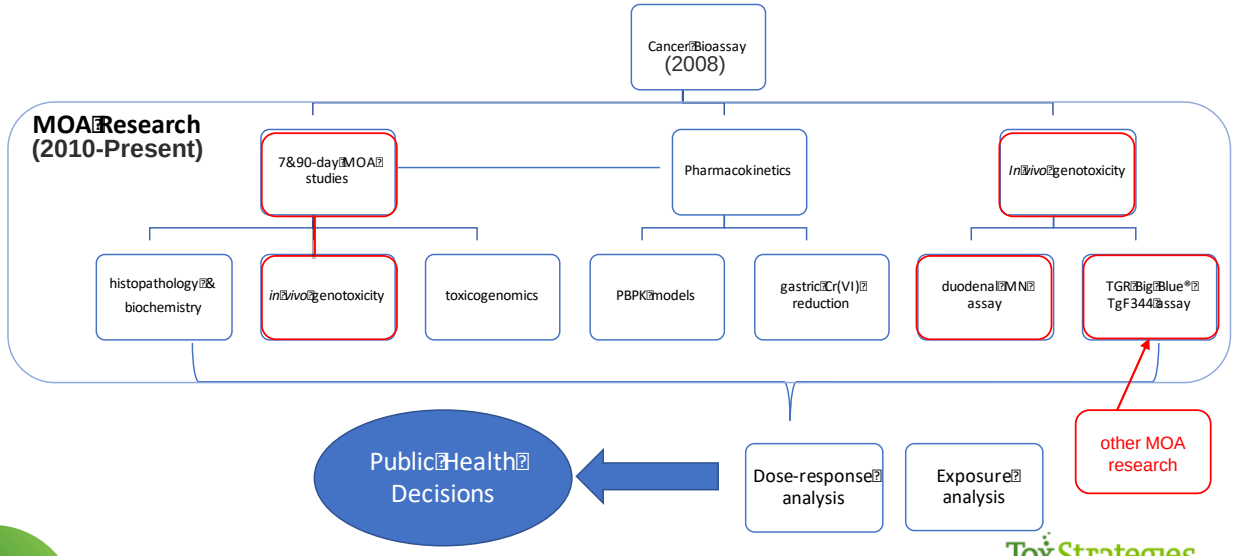
In this cross section of a mouse exposed to 180 ppm Cr(VI) for 90 days, XRF imaging reveals the presence of calcium (Ca) and sulfur (S) fluorescence throughout the crypts and villi (Thompson *et al.*, 2015a). In contrast, Cr(VI) is readily detected at villous tips and is nearly absent in the crypts. This indicates that toxicity most likely occurs in the villi, and not in the crypts or stem cells near the base of the crypt. Critically, this localization is consistent with the histopathology shown earlier indicating healthy elongating crypts, and blunting of the villi. These data also inform results from genotoxicity testing.

Evidence for Cytotoxicity/Regenerative Hyperplasia MOA

- Significant duodenal “diffuse epithelial hyperplasia” observed in mice of the NTP 13-wk and 2-year bioassay (NTP, 2007,2008)
- Neither NTP assay reported DEH or intestinal tumors in rats
- DEH also observed after only one week of exposure (Thompson et al., 2011)
- Phenotype for Cr(VI) is similar to captan and folpet
- Suggests a common cytotoxicity-regenerative hyperplasia MOA for Cr(VI)-induced intestinal cancers

What about genotoxicity?

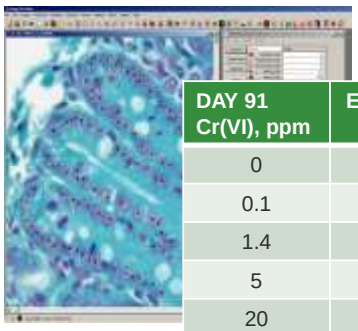
In Vivo Target Tissue Genotoxicity Studies



ToxStrategies

Cr(VI) Does Not Increase Micronuclei in Mouse Crypts

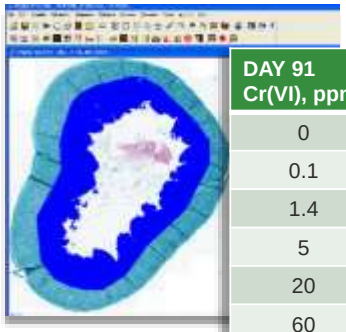
Intact Crypts



DAY 91 Cr(VI), ppm	Enterocytes	MN, KN
0	1921	0, 0
0.1	1707	0, 4*
1.4	1825	0, 0
5	1420	0, 0
20	2386	0, 0
60	2746	0, 0
180	3194	0, 0
O'Brien et al. (2013) Mut Res		

*3 observed in one animal

Full Sections



DAY 91 Cr(VI), ppm	Crypts MN, KN	Villi MN, KN
0	2, 0	1, 0
0.1	2, 1	1, 1
1.4	1, 0	2, 0
5	1, 0	0, 0
20	0, 1	2, **5**
60	0, 1	**9, 6**
180	0, 0	**9, 25**
O'Brien et al. (2013) Mut Res		

Note: bolded values are statistically significant

ToxStrategies

26

Using tissues from our original 90-day study in mice, target tissue *in vivo* genotoxicity was assessed for micronucleus induction and increased mutant frequency (O'Brien *et al.*, 2013). Stained tissue sections were digitized and scored by two methods: 1) the traditional analysis of intact crypts, and 2) by scoring entire sections in regions of crypts and villi. No increases in MN were noted in crypts; however, damaged nuclei were present in the villi—particularly around the tips.

Cr(VI) did not Increase *kras* Mutant Frequency in Mouse Duodenum

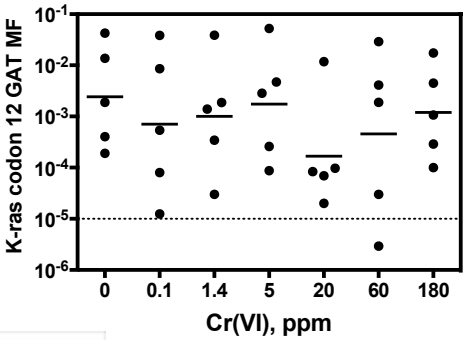
- No mutation data from intestinal tumors in the NTP Cr(VI) cancer bioassay
- K-ras selected b/c implicated in intestinal carcinogenesis
- Mutations often occur in codon 12
  - GGT→ GAT: spontaneous mutation; sometimes elevated with other K-ras mutations
  - K-ras^{G12D} can increase proliferation in mouse intestine
- Sensitive ACB-PCR assay
  - B6C3F1 mice exposed to Cr(VI) for 90 days
  - Codon 12 GAT mutations measured in scraped duodenal mucosa

ToxStrategies

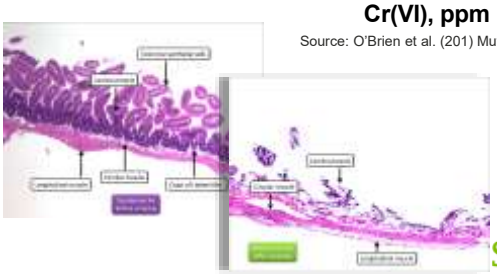
Cr(VI) did not Increase *kras* Mutant Frequency in Mouse Duodenum

- No r… …mors in th…
- K-ra… inte…
- Muta…
  - GGT→ GAT: spontaneous mutation; sometimes elevated with other K-ras mutations
  - K-ras^{G12D} can increase proliferation in mouse intestine
- Sensitive ACB-PCR assay
  - B6C3F1 mice exposed to Cr(VI) for 90 days
  - Codon 12 GAT mutations measured in scraped duodenal mucosa

Despite high MF, small intestine tumors rare among NTP studies.



Source: O'Brien et al. (201) Mut Res 754, 15-21.



Strategies

28

The potential for *in vivo* mutation was assessed in kras codon 12 by the ACB-PCR method. Unlike the MN assay, which focuses heavily on the crypt, mutation analysis was conducted in scraped intestinal mucosa containing crypts and villi. Kras codon 12 was selected, in part, because it has been implicated in intestinal carcinogenesis. Kras mutant frequency (MF) was not elevated in any treatment groups (O'Brien *et al.*, 2013), although the data might suggest that here is a high background mutation rate in kras codon 12 in B6C3F1 mice.

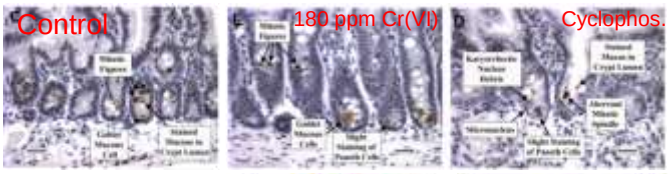
Follow Up 7-Day GLP MN Study

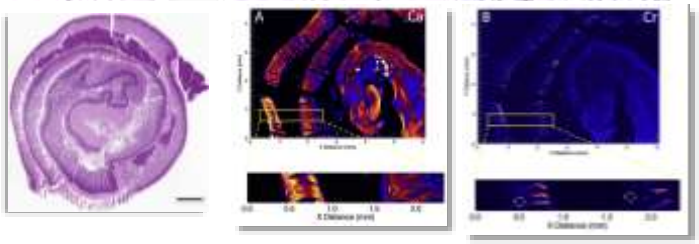
Cr did not increase MN

Cr did not increase y-H2AX

Cr localized to villi

Cr(VI), ppm	Enterocytes	Crypts	Cells/Crypt	MN	KN
0	6694	171	39.3	4	0
1.4	3159	77	41.0	1	0
21	3946	76	51.9	1	1
180	5161	77	67.1	0	0
Cyclophos.	3447	87	39.3	30	5



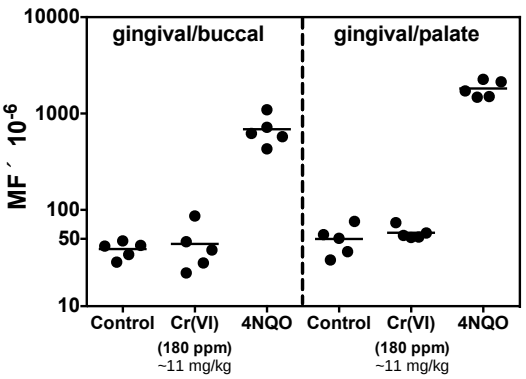


29

After O'Brien et al. (2013), a new 7-day GLP MN assay was conducted at BioReliance (Thompson *et al.*, 2015b). This study used 'Swiss roll' preps to better analyze crypts for MN. Synchrotron-based XRF mapping was again conducted to visualize the location of chromium in tissue. In addition, y-H2AX immunostaining was conducted to help visualize abnormal nuclei. Exposure to Cr(VI) caused a dose-dependent increase in cell proliferation (as measured by enterocytes per crypt) without increase in MN formation or aberrant y-H2AX staining. In contrast the positive control cyclophosphamide increased MN and aberrant y-H2AX immunostaining without effecting cell proliferation. XRF imaging revealed the presence of chromium in villi but not crypts.

TGR Mutation Assay in Big Blue® TgF344 Rats

- Transgenic rodent (TGR) in vivo mutagenicity assays can detect mutations in most all tissues
- Chose to run Big Blue rat to address the oral tumors in rats and the intestinal tumors in mice
  - XRF maps and pharmacokinetic data indicate relatively high levels of Cr(VI) in rats exposed to 180 ppm



Source: Thompson et al. 2015 EMM 56, 621-628.

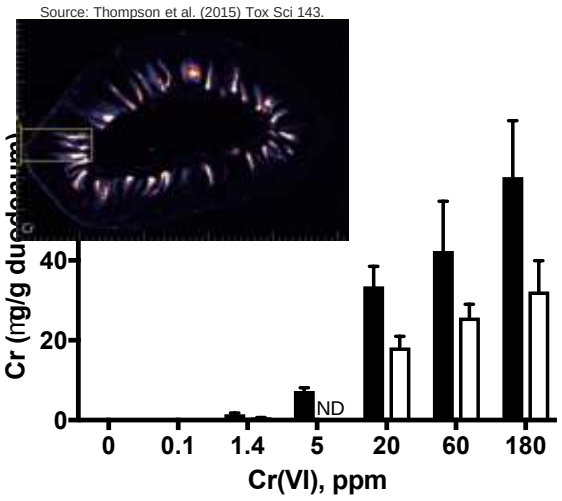
30

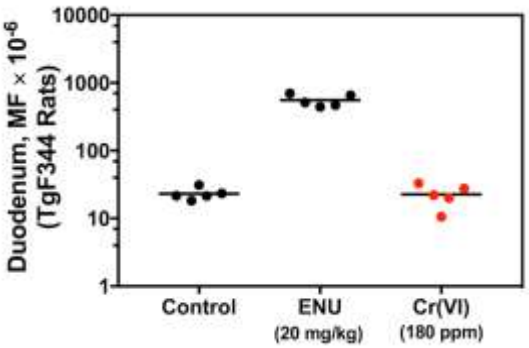
ToxStrategies

To further examine the potential for *in vivo* mutational events, a GLP Big Blue *in vivo* transgenic rodent (TGR) mutation assay was carried out in TgF344 rats. Big Blue TgF344 rats were selected, in part, to examine the potential for a mutagenic MOA for the oral cavity tumors in F344 rats at 180 ppm Cr(VI) in the NTP (2008) bioassay. Because the Big Blue model had not been previously qualified for examining mutant frequency in the oral cavity, we first worked with BioReliance to qualify this target tissue (Young *et al.*, 2015). After successful qualification, an in vivo mutation assay was conducted informed by OECD Test Guideline 488. Mutant frequency was not elevated in the oral cavity of TgF344 rats exposed to 180 ppm Cr(VI) (Thompson *et al.*, 2015c).

TGR Mutation Assay in Duodenum of Big Blue® TgF344 Rats

Source: Thompson et al. (2015) Tox Sci 143.





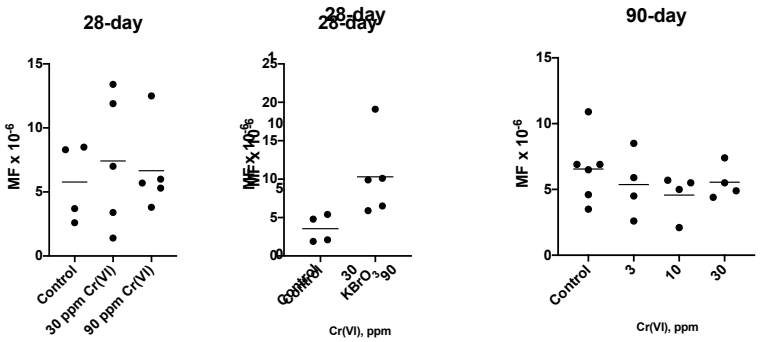
31

ToxStrategies

Because previous data collected demonstrated high levels of chromium in the rat duodenum following Cr(VI) exposure (Thompson *et al.*, 2012), it is reasonable to consider MF data from the rat duodenum informative for MOA even though rats did not develop intestinal tumors. Indeed, the point of running TGR assays is usually to be predictive of human health risk in the absence of carcinogenicity data. Mutant frequency was not elevated in the duodenum of TgF344 rats exposed to 180 ppm Cr(VI) (Thompson *et al.*, 2017).

Cr(VI) did Not Significantly Increase MF in Mouse Duodenum After 28-90 Days of Exposure

Mutant Frequency is not Increased in Mice Orally Exposed to Sodium Dichromate



32

ToxStrategies

A group of Japanese researchers conducted a TGR *in vivo* mutation assay in *gpt* delta mice (Aoki *et al.*, 2019). It seems that this work was conducted, in part, to support an ongoing assessment of Cr(VI) in Japan (FSCJ, 2019). Exposure of these mice to Cr(VI) did not significantly increase MF, and Aoki et al. concluded that the results were negative.

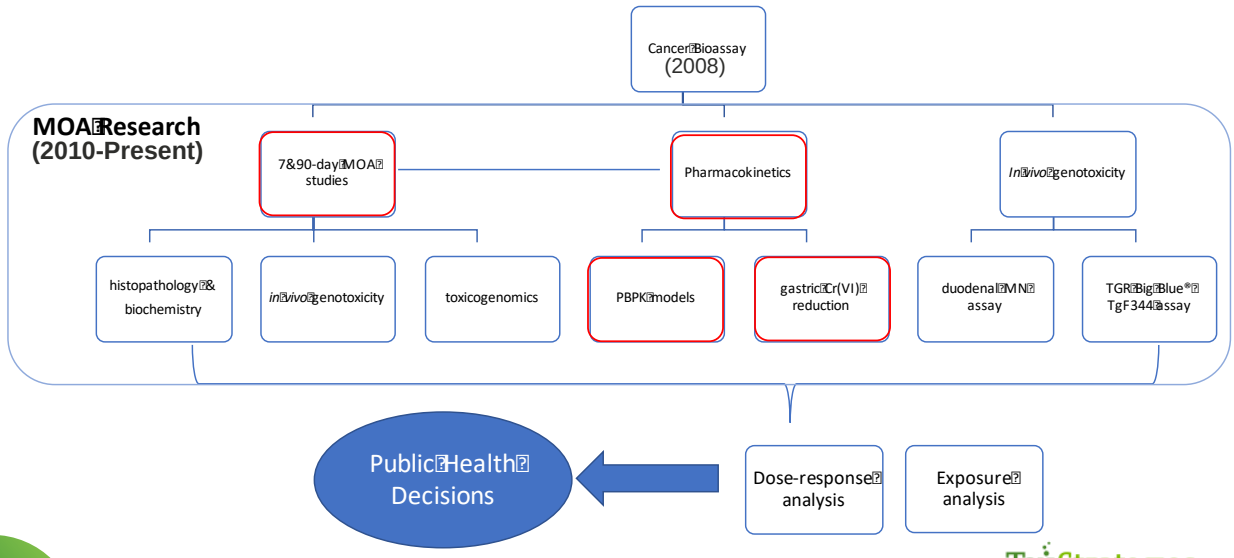
Evidence Against Target Tissue Genotoxicity

- MN formation in the crypt is negative in several Cr(VI) assays
- Mutant frequency is not elevated in the TgF344 rat oral mucosa or duodenum after exposure to 180 ppm Cr(VI)
- Mutant frequency is not elevated in the duodenum of male *gpt* delta mice exposed to up to 90 ppm Cr(VI)
- Data are consistent with XRF imaging
- Suggests a non-mutagenic MOA for Cr(VI)-induced intestinal cancers

Is there evidence of pharmacokinetic thresholds?

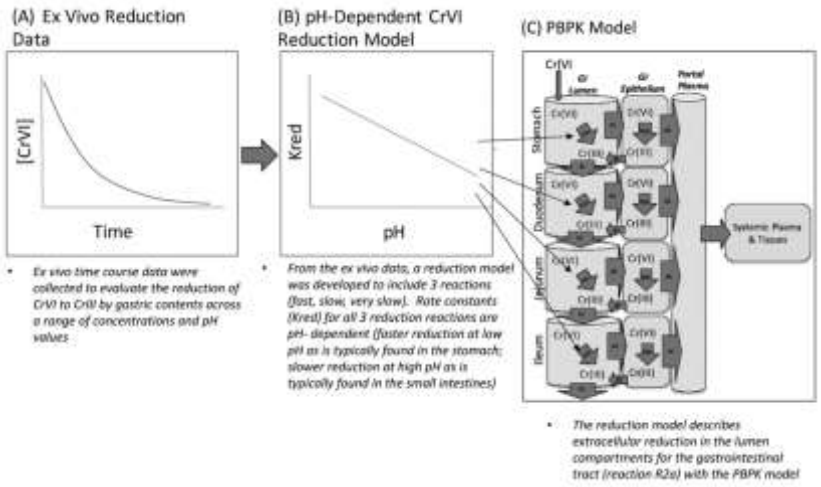
ToxStrategies

Cr(VI) Pharmacokinetic Studies



ToxStrategies

PBPK Models for Cr(VI): Data & Model Structure



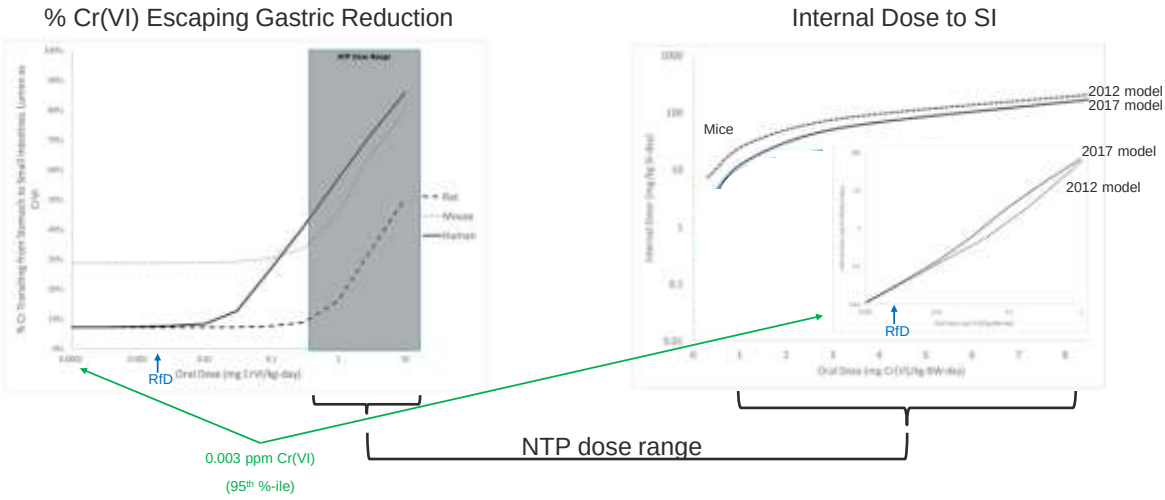
- *Ex vivo time course data were collected to evaluate the reduction of CrVI to CrIII by gastric contents across a range of concentrations and pH values*
- *From the ex vivo data, a reduction model was developed to include 3 reactions (fast, slow, very slow). Rate constants (Kred) for all 3 reduction reactions are pH- dependent (faster reduction at low pH as is typically found in the stomach; slower reduction at high pH as is typically found in the small intestines)*
- *The reduction model describes extracellular reduction in the lumen compartments for the gastrointestinal tract (reaction R2a) with the PBPK model*

35

ToxStrategies

The PBPK model (Kirman *et al.*, 2017) begins with data on the reduction of Cr(VI) to Cr(III) by mouse, rat, and human gastric fluid. This data was then used to develop a reduction model representing the extracellular reduction of Cr(VI) to inert Cr(III), i.e. detoxification of Cr(VI). These data then feed into a model of the amount of Cr(VI) reduced within the lumen of each segment of the intestine (duodenum, jejunum, ileum) and ultimately a PBPK describing the tissue levels in the portal of entry (POE) and systemic compartments.

Nonlinearities in Pharmacokinetics



Kirman et al. (2017)

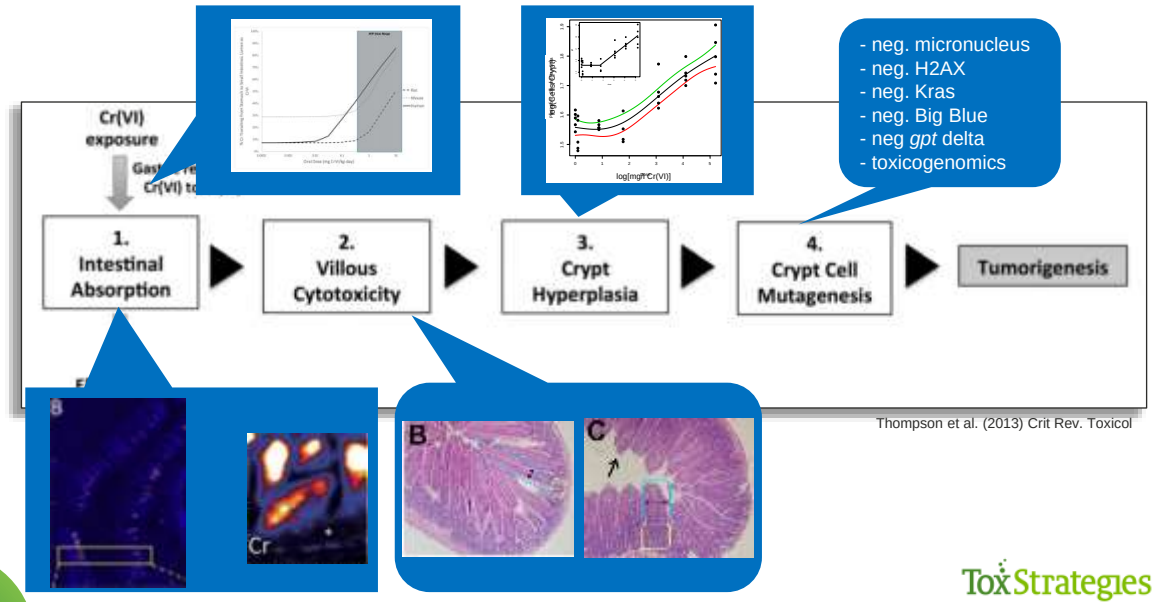
ToxStrategies

36

Under the exposure concentrations in the NTP (2008) bioassay, nonlinear toxicokinetics are predicted to occur in rats and mice due to depletion of reducing equivalents in gastric fluid. At doses <0.1 mg/kg, the percentage of Cr reaching the small intestines as Cr(VI) is greater in mice than in rats and humans due primarily to the shorter transit time for gastric contents in mice. This shorter transit time allows less time for Cr(VI) reduction to occur. As reducing equivalents become depleted at higher doses, the percentage of Cr leaving the stomach and reaching the small intestines as Cr(VI) increases. Nonlinear toxicokinetics are predicted by the PBPK model to occur in humans at doses >0.01 mg/kg-day, in mice and rats at doses >0.1 mg/kg-day. The difference in inflection points across species is largely due to differences in reduction capacity for the fast reduction portion of the reduction model.

The plot on the right shows the dose-dependent increase in Cr(VI) entering the SI over the dose range in the NTP bioassay. The inset shows the model-predicted dose-dependent increase in Cr(VI) entering the human SI at environmental levels. At the current RfD of 0.003 mg/kg-day, the amount of Cr(VI) entering the SI is a fraction of the internal dose required to induce tumors in mice. The 95th percentile Cr(VI) concentration in US water sources (0.003 ppm) is equivalent to a daily dose of 0.0001 mg/kg-day, obviously resulting in even less internal Cr(VI) exposure.

Non-mutagenic MOA for SI Tumors



Thompson et al. (2013) Crit Rev. Toxicol

37

Narrow Range of RfDs Generated for Cr(VI)

Author	RfD or TDI (mg/kg-day)	Drinking Water (ppb)	Data Used
Thompson et al. (2018)	0.003* (same as current IRIS value)	Current MCL	NTP (SI hyperplasia) PBPK Models (dose metric) MOA Research (threshold extrapolation)
Health Canada (2016)	0.0022	50 (same as before)	NTP (SI hyperplasia) PBPK Models (dose metric) MOA Research (threshold extrapolation)
TCEQ (2016)	0.003	≅ 100	NTP (SI hyperplasia) PK Data (dose metric) MOA Research (threshold extrapolation)
Food Safety Commission of Japan (2019)	0.001	30-60	NTP (SI hyperplasia) MOA Research (threshold extrapolation)
WHO (2019, Draft)	--	50 (same as before)	MOA Research (retain existing value)

*no other endpoints in NTP (2008) bioassay (e.g., liver inflammation) resulted in lower RfD values

38

ToxStrategies

Based on updated PBPK models for Cr(VI), a risk assessment for providing an oral RfD for Cr(VI) was conducted (Thompson *et al.*, 2018). Potentially adverse effects from Cr(VI) exposure in the NTP (2008) bioassay were modeled using dose metrics suitable for point of contact in the intestine and systemic delivery for other tissues (e.g., liver). The RfD for intestinal hyperplasia and liver inflammation resulted in the lowest RfD values of 0.003 mg/kg-day. This value is identical to the current RfD in IRIS; however, the confidence in the updated RfD is improved due to the use of a gold standard NTP cancer bioassay, MOA information, PBPK models, and BMD modeling. All of the recently published values fall within a very narrow range—despite differences in the derivation of the safety values.

MOA Summary

- The scientific weight of evidence strongly favors a non-mutagenic MOA supporting nonlinear risk assessment approaches
- Several authoritative bodies have concluded (with varying degrees of systematic evaluation) that the MOA for oral tumors observed in the NTP (2008) bioassay is likely non-mutagenic and therefore have developed regulatory values using nonlinear methods
- Default low-dose linear extrapolation leads to the unsupported conclusion that current environmental exposures to Cr(VI) pose a health risk

Remaining Issues

Tox Strategies

Two New Manuscripts Relevant to Cr(VI)

Adverse Outcome Pathway (AOP) for small intestine cancer

- Accepted at Critical Reviews in Toxicology
- Coauthors: Sam Cohen, Charles Wood, John Cullen, Elliot Gordon, Virunya Bhat, Mark Harris, Deborah Proctor, and Chad Thompson
- Describes the initial development of an AOP for intestinal cancer in mice
  - Builds on knowledge from Cr(VI), captan, and folpet databases
  - Informed by other intestinal maladies

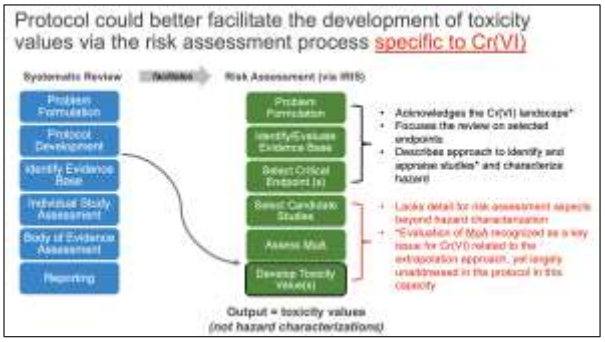
Use of 10 Key Characteristics of Cancer to Evaluate Alternative MOAs

- Under review at Toxicological Sciences
- Coauthors: Grace Chappell, Daniele Wikoff, and Chad Thompson
- Article evaluates data for key characteristics of carcinogens (KCC) not related to the two proposed/published MOAs: cytotoxicity/regenerative hyperplasia and mutagenic MOA

ToxStrategies

Systematic Review(SR) and Assessment for Cr(VI)?

- Commend EPA's continued evolution of applying SR for risk assessment
- ToxStrategies provided comments during April 2019 webinar regarding the SR protocol for Cr(VI) (see right)
- Unclear how Cr(VI) assessment methods have been modified based on public input to the protocol
- Unclear how Cr(VI) assessment methods will compare to those in the anticipated IRIS Handbook



Slide 4: Wikoff, Public Comments, 23Apr19

42

ToxStrategies

We commend USEPA's leadership in the application of systematic review in chemical risk assessment. In April of 2019, ToxStrategies provided comments on the systematic review protocol for Cr(VI). Therein, a logical and pragmatic use of systematic review was described for the initial stages of risk assessment – specifically, the reliance of previous assessment as well as an independent search to identify literature and characterize hazard. However, there was a lack of detail for how systematic review will be applied to risk assessment aspects beyond these initial stages, and how mechanistic data will be evaluated beyond identification via the key characteristics. As a result, the protocol was seemingly not aligned with Agency's own guidance on MOA in the cancer guidelines – despite MOA being a critical component of risk assessment.

As practitioners of systematic review, we certainly recognize the challenge in applying EBT methods to mechanistic data. We specifically identify and discuss a number of these challenges in our evaluation of KCC data for Cr(VI) in a new manuscript that is currently under peer review. In this assessment, we utilized the studies tagged as "mechanistic" in HAWC by the USEPA and reviewed the data to determine the potential for alternative MOAs. This exercise highlighted potential utilities in a broad organizational approach such as KCC, though also highlights the challenges in not having a structured evidence-to-decision method identified a priori for these types of data. That is, mechanistic data associated with any of the KCCs must be evaluated in context of specific chemical and tumor (or adverse outcome) under evaluation. We found that when mechanistic data were evaluated with the anchor of SI tumors in mice, no plausible alternative MOAs were identified to the two MOAs that have been proposed: mutagenic and cytotoxicity/regenerative hyperplasia. Among these two MOAs, that available data provide greater weight to the latter.

Given the importance of utilizing systematic review in a "fit for purpose" manner through focus on studies that inform quantitative methods for setting standards (e.g., targeted research on tumors), we are wondering if EPA can provide an update regarding methodological updates relating to how MOA will be evaluated using systematic review?

And/or if the methods applied for Cr(VI) are similar to that which will be described in the anticipated IRIS handbook for systematic review? And if the IRIS Handbook will more specifically address MOA?

Remaining Topics

- **IRIS Schedule Outlook**
- **Outstanding Issues or Questions**

References

See notes

ToxStrategies

Aoki, Y., Matsumoto, M., Matsumoto, M., Masumura, K. and Nohmi, T. (2019). Mutant frequency is not increased in mice orally exposed to sodium dichromate. *Food Safety* **7**, 2-10.

Cohen, S. M., Gordon, E. B., Singh, P., Arce, G. T. and Nyska, A. (2010). Carcinogenic mode of action of folpet in mice and evaluation of its relevance to humans. *Crit Rev Toxicol* **40**, 531-45.

FSCJ (2019). Risk Assessment Report Hexavalent Chromium (Beverages). *Food Safety Commission of Japan* **7**, 56-57.

Health Canada (2016). Guidelines for Canadian Drinking Water Quality: Guideline Technical Document — Chromium. (H. E. a. C. S. B. Water and Air Quality Bureau, Ed.^ Eds.). Health Canada, Ottawa, Ontario. .

Kirman, C. R., Suh, M., Proctor, D. M. and Hays, S. M. (2017). Improved physiologically based pharmacokinetic model for oral exposures to chromium in mice, rats, and humans to address temporal variation and sensitive populations. *Toxicol Appl Pharmacol* **325**, 9-17.

Mackenzie, R. D., Byerrum, R. U., Decker, C. F., Hoppert, C. A. and Langham, R. F. (1958). Chronic toxicity studies. II. Hexavalent and trivalent chromium administered in drinking water to rats. *AMA Arch Ind Health* **18**, 232-4.

NTP (2007). National Toxicology Program technical report on the toxicity studies of sodium dichromate dihydrate (CAS No. 7789-12-0) administered in drinking water to male and female F344/N rats and B6C3F1 mice and male BALB/c and *am3*-C57BL/6 mice. *NTP Toxicity Report Series Number 72, NIH Publication No. 07-5964*.

NTP (2008). National Toxicology Program technical report on the toxicology and carcinogenesis studies of sodium dichromate dihydrate (CAS No. 7789-12-0) in F344/N rats and B6C3F1 mice (drinking water studies), NTP TR 546. *NIH Publication No. 08-5887*.

O'Brien, T. J., Ding, H., Suh, M., Thompson, C. M., Parsons, B. L., Harris, M. A., Winkelman, W. A., Wolf, J. C., Hixon, J. G., Schwartz, A. M., Myers, M. B., Haws, L. C. and Proctor, D. M. (2013). Assessment of K-Ras mutant frequency and micronucleus incidence in the mouse duodenum following 90-days of exposure to Cr(VI) in drinking water. *Mutat Res* **754**, 15-21.

OEHHA (2011). Final Technical Support Document on Public Health Goal for Hexavalent Chromium in Drinking Water. *Pesticide and Environmental Toxicology Branch, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency*.

Stern, A. H. (2010). A quantitative assessment of the carcinogenicity of hexavalent chromium by the oral route and its relevance to human exposure. *Environ Res* **110**, 798-807.

Stout, M. D., Herbert, R. A., Kissling, G. E., Collins, B. J., Travlos, G. S., Witt, K. L., Melnick, R. L., Abdo, K. M., Malarkey, D. E. and Hooth, M. J. (2009). Hexavalent chromium is carcinogenic to F344/N rats and B6C3F1 mice after chronic oral exposure. *Environ Health Perspect* **117**, 716-22.

TCEQ (2016). Hexavalent Chromium Oral Reference Dose: Development Support Document (Final).

Thompson, C. M., Proctor, D. M., Suh, M., Haws, L. C., Hebert, C. D., Mann, J. F., Shertzer, H. G., Hixon, J. G. and Harris, M. A. (2012). Comparison of the effects of hexavalent chromium in the alimentary canal of F344 rats and B6C3F1 mice following exposure in drinking water: implications for carcinogenic modes of action. *Toxicological Sciences* **125**, 79-90.

Thompson, C. M., Seiter, J., Chappell, M. A., Tappero, R. V., Proctor, D. M., Suh, M., Wolf, J. C., Haws, L. C., Vitale, R., Mittal, L., Kirman, C. R., Hays, S. M. and Harris, M. A. (2015a). Synchrotron-Based Imaging of Chromium and gamma-H2AX Immunostaining in the Duodenum Following Repeated Exposure to Cr(VI) in Drinking Water. *Toxicol Sci* **143**, 16-25.

Thompson, C. M., Wolf, J. C., Elbekai, R. H., Paranjpe, M. G., Seiter, J. M., Chappell, M. A., Tappero, R. V., Suh, M., Proctor, D. M., Bichteler, A., Haws, L. C. and Harris, M. A. (2015b). Duodenal crypt health following exposure to Cr(VI): Micronucleus scoring, gamma-H2AX immunostaining, and synchrotron X-ray fluorescence microscopy. *Mutation research. Genetic toxicology and environmental mutagenesis* **789-790**, 61-6.

Thompson, C. M., Young, R. R., Suh, M., Dinesdurage, H. R., Elbekai, R. H., Harris, M. A., Rohr, A. C. and Proctor, D. M. (2015c). Assessment of the mutagenic potential of Cr(VI) in the oral mucosa of Big Blue((R)) transgenic F344 rats. *Environ Mol Mutagen* **56**, 621-8.

Thompson, C. M., Young, R. R., Dinesdurage, H., Suh, M., Harris, M. A., Rohr, A. C. and Proctor, D. M. (2017). Assessment of the mutagenic potential of hexavalent chromium in the duodenum of big blue(R) rats. *Toxicol Appl Pharmacol* **330**, 48-52.

Thompson, C. M., Kirman, C. R., Hays, S. M., Suh, M., Harvey, S. E., Proctor, D. M., Rager, J. E., Haws, L. C. and Harris, M. A. (2018). Integration of mechanistic and pharmacokinetic information to derive oral reference dose and margin-of-exposure values for hexavalent chromium. *J Appl Toxicol* **38**, 351-365.

U.S. EPA (2017). Data Summary of The Third Unregulated Contaminant Monitoring Rule (UCMR3). *EPA 815-S-17-001*.

WHO (2019). Chromium in Drinking Water: Draft Background Document for Development of WHO Guidelines for Drinking-water quality.

Young, R. R., Thompson, C. M., Dinesdurage, H. R., Elbekai, R. H., Suh, M., Rohr, A. C. and Proctor, D. M. (2015). A robust method for assessing chemically induced mutagenic effects in the oral cavity of transgenic Big Blue(R) rats. *Environ Mol Mutagen* **56**, 629-36.