# Mode of Action & Toxicity Criteria for Oral Exposure to Hexavalent Chromium

Chad Thompson

North Carolina Science Advisory Board

April 1, 2019

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# Funding Acknowledgements

This work was funded by Hexavalent Chromium Panel of the American Chemistry Council (ACC), and the Electric Power Research Institute (EPRI), an independent nonprofit 501(c)3 organization that funds external research at a number of universities and institutes worldwide.

# Presentation Outline

- Background
- Results from targeted MOA research
- Implications for risk assessment

# Previous Presentations to NC SAB on Cr(VI)

- June 2018 – NJDEP & TCEQ
  - NJDEP – stated presentation is a "summary of CSF derivation…contained in the 2010 publication…there is really no new information other than what was presented in the paper…"
    - Developed a cancer slope factor
  - TCEQ – no date limitation on analysis
    - Developed an RfD protective of cancer and non-cancer effects

- August 2018 – OEHHA & Health Canada
  - OEHHA – stated "…I will be speaking about the PHG that was established in 2011"
    - OEHHA is reviewing/updating the PHG but did not discuss the ongoing work
    - Developed a cancer slope factor (similar to NJDEP)
  - Health Canada – no date limitation on analysis
    - Developed an RfD protective of cancer and non-cancer effects

# Existing IRIS File for Chromium (1998-present)

**I.A.1. Oral RfD Summary**

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



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# NTP Cr(VI) and Cr(III) Bioassays (2008)

**NTP Cr(VI) drinking water study**

- 5 to 180 ppm in drinking water
- Rare tumors appeared late in the study

  Mice: adenomas and carcinomas of SI (≥30 ppm)

  Rats: SCC in oral cavity (180 ppm)

**NTP Cr(III) 2 year feed study**

- 2,000 to 50,000 ppm in diet
- No significant effects in either species

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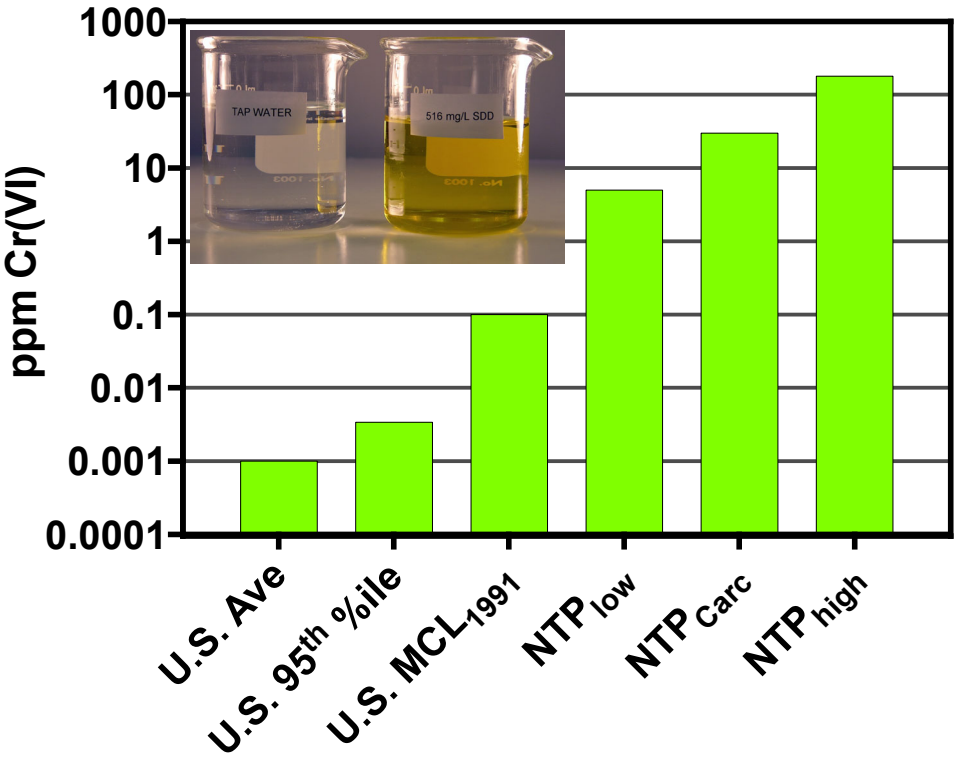


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# Cr(VI) MOA Research Project

**Replicated aspects of NTP Cr(VI) study**

- Same strains (B6C3F1 mice, F344 rats)
- Same doses, plus two lower doses (including MCL)
- Data collected after 7 and 90 days of exposure

**Specifically investigated target tissue of small intestine and oral mucosa**

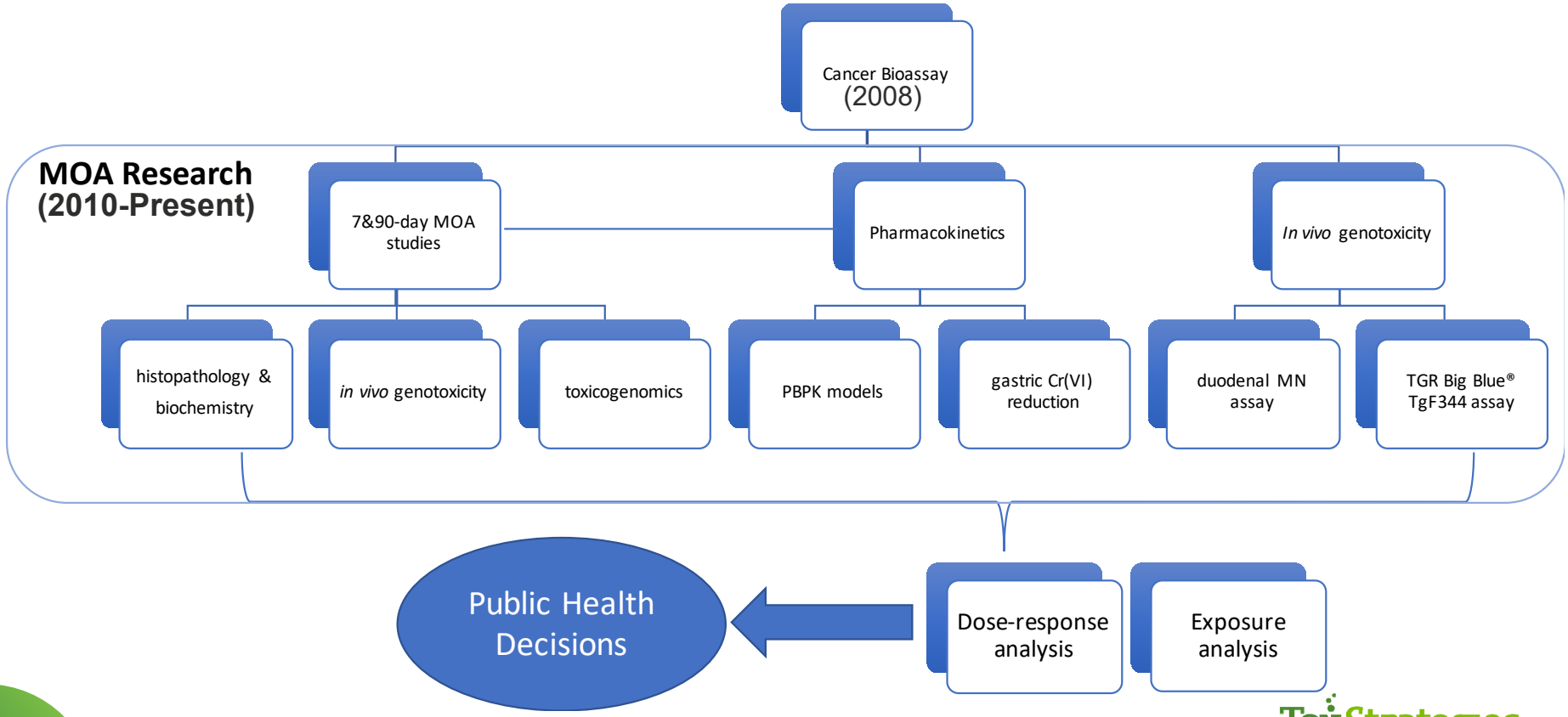
- Histopathology
- *In vivo* genotoxicity
- Toxicogenomics
- Biochemistry
- *In vitro* genotoxicity

**Evaluated toxicokinetics**

- Measured rates and capacity of Cr(VI) reduction to Cr(III) in human and rodent stomach contents
- Developed Physiologically-based Pharmacokinetic (PBPK) Models

**Studies were designed to inform risk assessment**

# Overview of Research Program



# Collaborators and Co-authors on MOA Studies

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Travis J. O'Brien

Jennifer M. Seiter
Mark A. Chappel

Timothy R. Zacharewski
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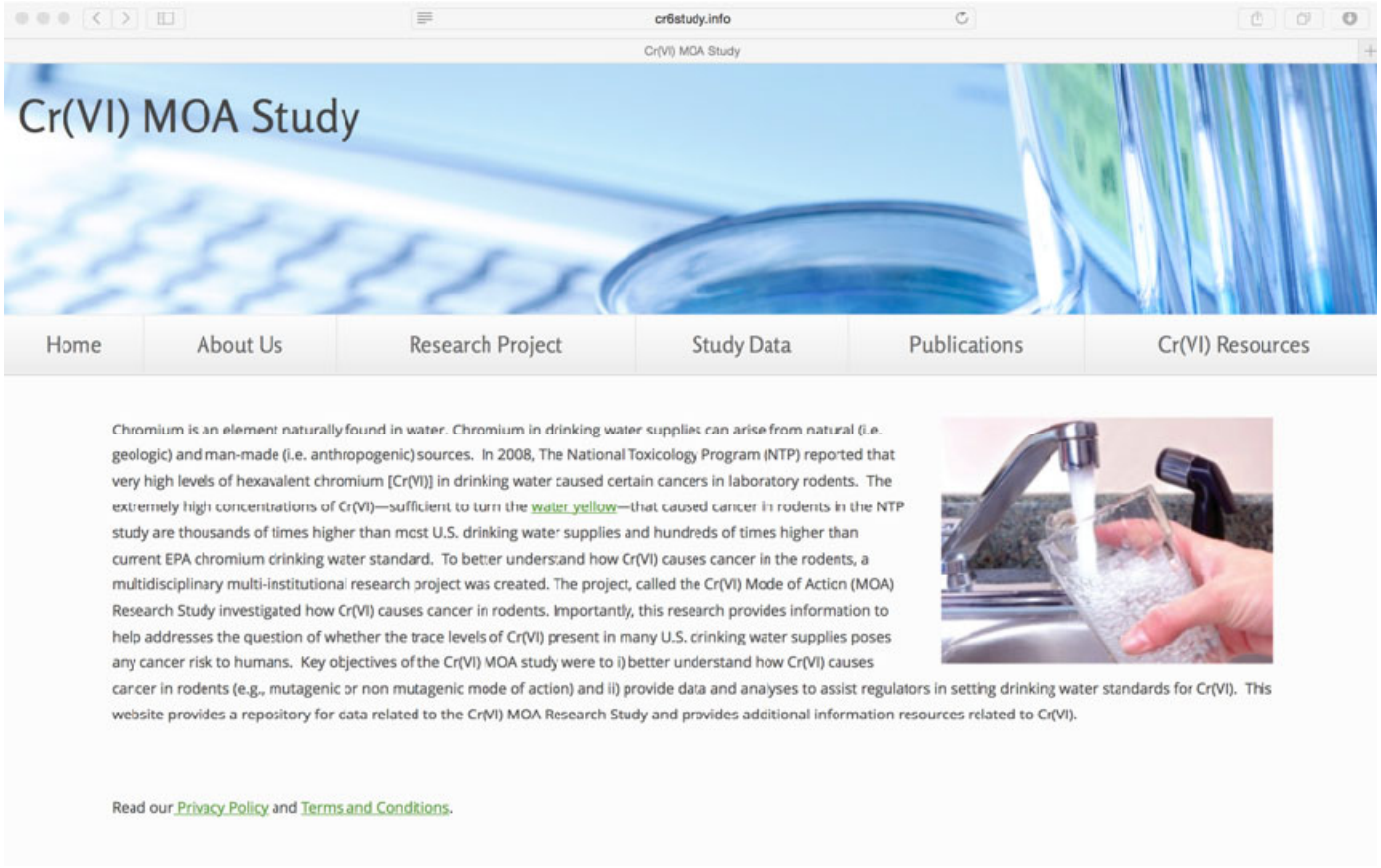
Jeffrey C. Wolf

# Study Transparency: Data Publically Available



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# Genotoxic Potential of Cr(VI)

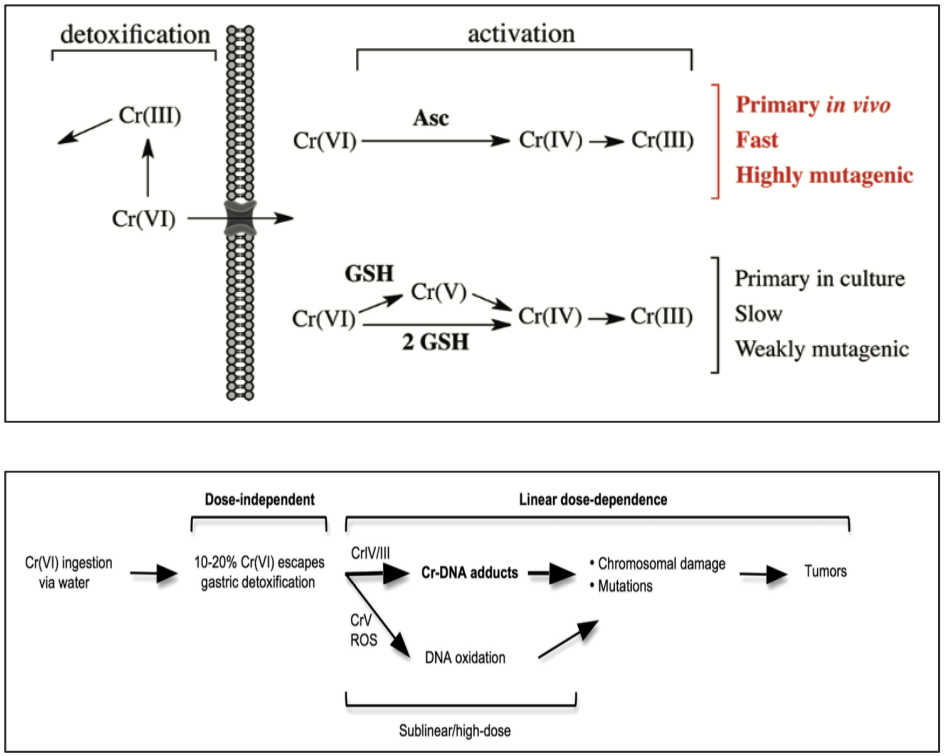


Figure Source: Zhitkovich et al. 2011, Chem Res Toxicol. 24, 1617.

**Expert panel member's comments on EPA draft Cr(VI) risk assessment:**

There is no doubt that Cr(VI) can be forced to be genotoxic and "mutagenic" under experimentally contrived systems and at high doses that evoke major amounts of cell death.

…in hindsight many of us "DNA damage and repair" scientists have come to appreciate several important factors: (i) DNA damage is only observed at very high dose that kill a lot of cells, (ii) Cr(VI) is at best a very weak "mutagen", requiring very high doses that kill most cells and experimental "backflips" to select for survivors, and

…(iii) what we thought was "mutagenesis" is actually selection for stochastic cell survivors of massive toxic insult.



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# MOA Analysis is Conducted for the Tumor, Not the Agent

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

**1.3.4 Dose-response Assessment**

- 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.

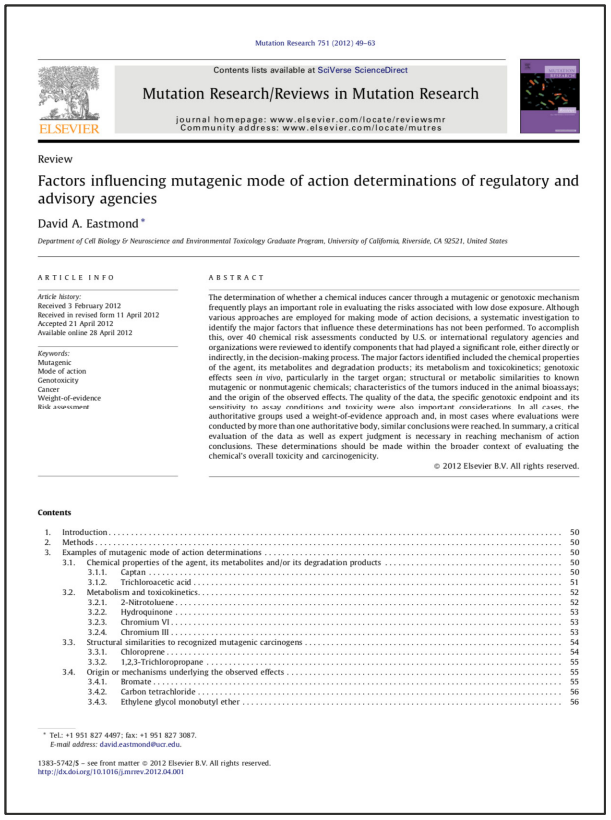
**2.4.3.1 Description of the Hypothesized Mode of Action**

- 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.

**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)

# Factors for Mode of Action Determinations



Mutation Research 751 (2012) 49–63

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Mutation Research/Reviews in Mutation Research

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Review

Factors influencing mutagenic mode of action determinations of regulatory and advisory agencies

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ABSTRACT

The determination of whether a chemical induces cancer through a mutagenic or genotoxic mechanism frequently plays an important role in evaluating the risks associated with low dose exposure. Although various approaches are employed for making mode of action decisions, a systematic investigation to identify the major factors that influence these determinations has not been performed. To accomplish this, over 40 chemical risk assessments conducted by U.S. or international regulatory agencies and organizations were reviewed to identify components that had played a significant role, either directly or indirectly, in the decision-making process. The major factors identified included the chemical properties of the agent, its metabolites and degradation products; its metabolism and toxicokinetics; genotoxic effects seen *in vivo*, particularly in the target organ; structural or metabolic similarities to known mutagenic or nonmutagenic chemicals; characteristics of the tumors induced in the animal bioassays; and the origin of the observed effects. The quality of the data, the specific genotoxic endpoint and its sensitivity to assay conditions and toxicity were also important considerations. In all cases, the authoritative groups used a weight-of-evidence approach and, in most cases where evaluations were conducted by more than one authoritative body, similar conclusions were reached. In summary, a critical evaluation of the data as well as expert judgment is necessary in reaching mechanism of action conclusions. These determinations should be made within the broader context of evaluating the chemical's overall toxicity and carcinogenicity.



**Contents**



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| Factors Influencing MOA Determinations |
|---|
| Chemical properties |
| Toxicokinetics |
| Structural similarities to other carcinogens |
| Nature of the tumors |
| Mutational spectrum |
| Origin of mechanisms |
| Understanding assays |
| Quality, quantity, & reproducibility |
| In vivo genotoxicity (especially in target organs) |
| Evidence for an alternative MOA |



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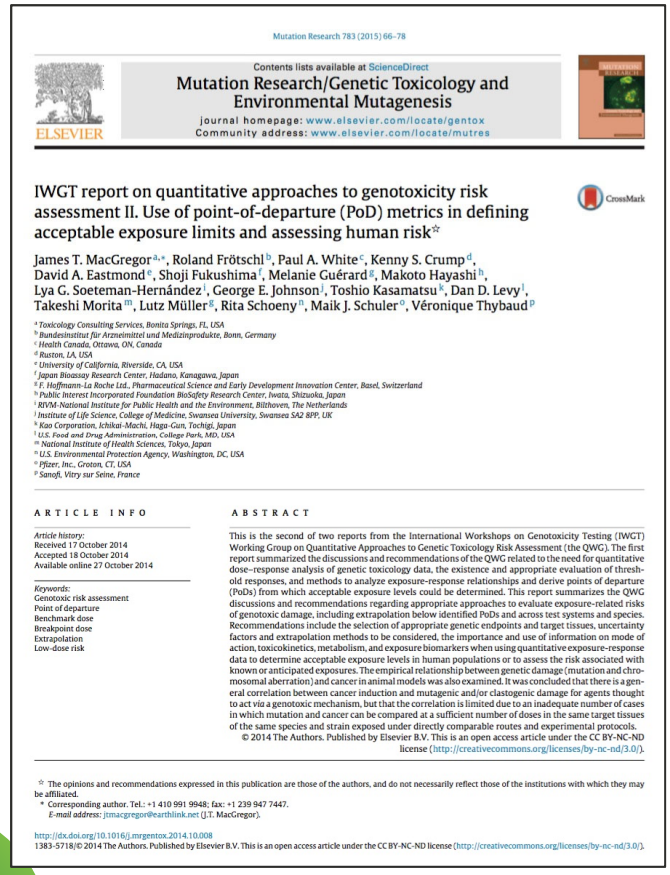
# *In Vivo* Blood and Bone Marrow MN Data for Cr(VI)

| NTP (2007) 90-day GLP Studies |
|---|
| B6C3F1, ≤88 ppm dw, M, (-) |
| B6C3F1, ≤350 ppm dw, M (-) |
| B6C3F1, ≤350 ppm dw, F (-) |
| BALB/c, ≤88 ppm dw, M, (-) |
| *Am3*-C57BL/6, ≤88 ppm dw, M, (+) |

dw, drinking water

# IWGT Recommendations for *In Vivo* Genotoxicity Assays



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IWGT report on quantitative approaches to genotoxicity risk assessment II. Use of point-of-departure (PoD) metrics in defining acceptable exposure limits and assessing human risk☆

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ABSTRACT

This is the second of two reports from the International Workshops on Genotoxicity Testing (IWGT) Working Group on Quantitative Approaches to Genetic Toxicology Risk Assessment (the QWG). The first report summarized the discussions and recommendations of the QWG related to the need for quantitative dose–response analysis of genetic toxicology data, the existence and appropriate evaluation of threshold responses, and methods to analyze exposure-response relationships and derive points of departure (PoDs) from which acceptable exposure levels could be determined. This report summarizes the QWG discussions and recommendations regarding appropriate approaches to evaluate exposure-related risks of genotoxic damage, including extrapolation below identified PoDs and across test systems and species. Recommendations include the selection of appropriate genetic endpoints and target tissues, uncertainty factors and extrapolation methods to be considered, the importance and use of information on mode of action, toxicokinetics, metabolism, and exposure biomarkers when using quantitative exposure-response data to determine acceptable exposure levels in human populations or to assess the risk associated with known or anticipated exposures. The empirical relationship between genetic damage (mutation and chromosomal aberration) and cancer in animal models was also examined. It was concluded that there is a general correlation between cancer induction and mutagenic and/or clastogenic damage for agents thought to act *via* a genotoxic mechanism, but that the correlation is limited due to an inadequate number of cases in which mutation and cancer can be compared at a sufficient number of doses in the same target tissues of the same species and strain exposed under directly comparable routes and experimental protocols.



☆ The opinions and recommendations expressed in this publication are those of the authors, and do not necessarily reflect those of the institutions with which they may be affiliated.

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1383-5718/

- ✓ Ideally conducted in a proliferative tissue
  - Bone marrow (hematopoietic)
  - Colon
  - **Small intestine (duodenum)**
- ✓ Ideally at site of carcinogenic action
  - **GI tract for Cr(VI)**
- ✓ Ideally in tissue with high dosimetry (e.g. site of contact)
  - **Duodenum for Cr(VI)**



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# Measured Chromium is Highest in Duodenum of Mice & Rats

Tissue total chromium concentrations (mg/kg) in mice exposed to Cr(VI) in drinking water for 90 days.

| Drinking water concentration, mg SDD/L (dose in mg Cr/kg day) | Oral* | Stomach | Duodenum | Jejunum | Ileum |
|---|---|---|---|---|---|
| 0 (0) | 0.25 ± 0.24 | 0.060 ± 0.019 | 0.017 ± 0.007 | 0.046 ± 0.044 | 0.020 ± 0.009 |
| 0.3 (0.024) | 0.18 ± 0.10 | 0.052 ± 0.023 | 0.056 ± 0.015 | 0.034 ± 0.021 | 0.014 ± 0.000 |
| 4 (0.32) | 0.21 ± 0.21 | 0.088 ± 0.016 | 1.5 ± 0.27 | 0.11 ± 0.052 | 0.042 ± 0.033 |
| 14 (1.1) | 0.66 ± 0.34 | 0.38 ± 0.086 | **7.3 ± 0.78** | 0.33 ± 0.29 | 0.13 ± 0.027 |
| 60 (4.6) | **3.7 ± 3.1** | **2.2** ± 0.27 | **33.5 ± 5.0** | **4.7 ± 3.3** | **0.92 ± 1.0** |
| 170 (11.6) | **4.1 ± 2.6** | **4.3 ± 0.64** | **42.4 ± 12.4** | **21.6 ± 14.8** | **1.8 ± 1.1** |
| 520 (30.9) | **7.9 ± 4.4** | **21.2 ± 1.6** | **60.9 ± 14.1** | **13.9 ± 6.9** | **2.3 ± 0.86** |

* $n = 5$; bolded values are significantly different from controls (Shirley's test, $p < 0.05$).

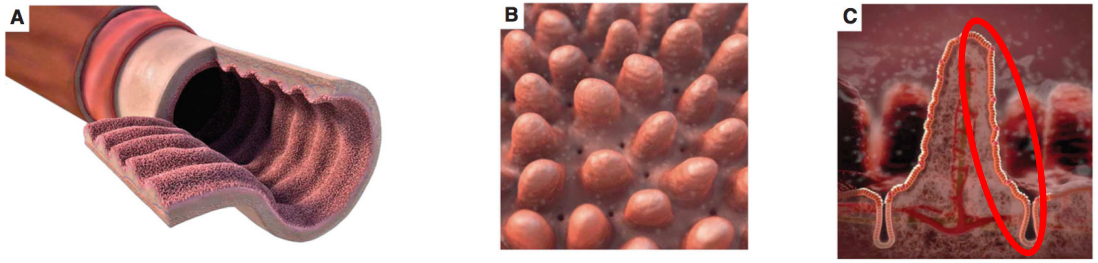
Tissue total chromium concentrations (mg/kg) in rats exposed to Cr(VI) in drinking water for 90 days.

| Drinking water concentration, mg SDD/L (dose in mg Cr/kg day) | Oral* | Stomach | Duodenum | Jejunum | Ileum |
|---|---|---|---|---|---|
| 0 (0) | 0.13 ± 0.16 | 0.15 ± 0.23 | 0.04 ± 0.02 | 0.03 ± 0.01 | 0.06 ± 0.04 |
| 0.3 (0.015) | 0.07 ± 0.07 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| 4 (0.21) | 0.05 ± 0.00 | 0.07 ± 0.06 | 0.49 ± 0.14 | 0.13 ± 0.14 | 0.04 ± 0.02 |
| 60 (2.9) | **1.0 ± 0.35** | 1.2 ± 0.50 | **18.2 ± 2.8** | **5.1 ± 3.7** | 0.85 ± 0.5 |
| 170 (7.2) | **2.1 ± 0.30** | **7.0 ± 2.4** | **25.7 ± 3.3** | **7.9 ± 7.7** | 1.6 ± 1.9 |
| 520 (20.5) | **5.0 ± 0.70** | **16.4 ± 5.3** | **32.2 ± 7.7** | **5.8 ± 3.0** | **1.2 ± 0.51** |

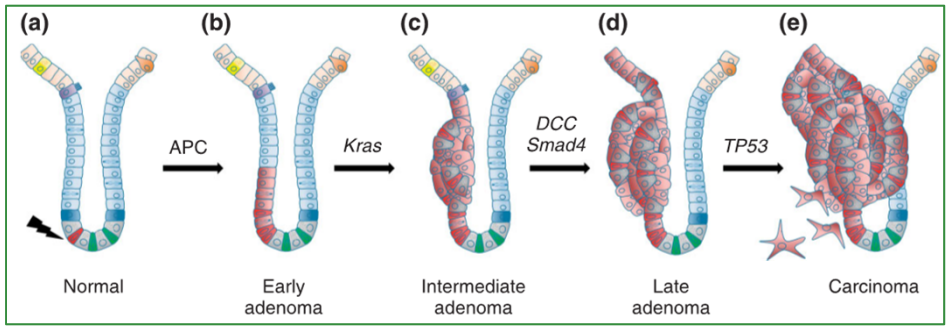
* $n = 5$; bolded values are significantly different from controls (Shirley's test, $p < 0.05$).

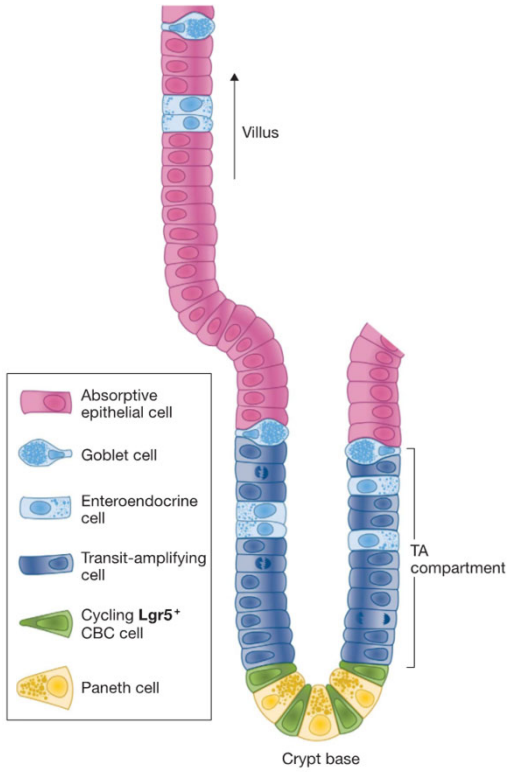
Source: Kirman et al. (2012) CBI. 200, 45.

# Small Intestine Structure and Carcinogenesis




## Model of Intestinal Cancer Initiation & Progression

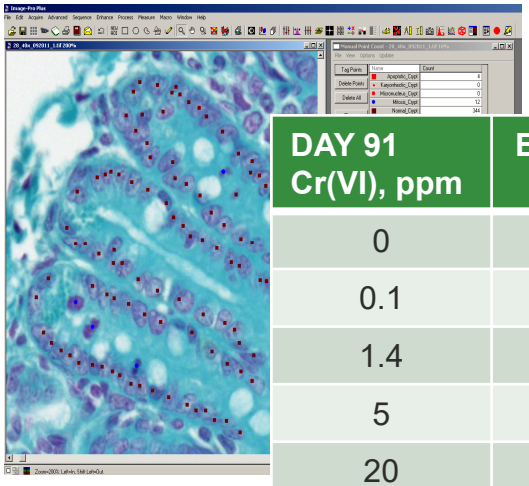



Sources: Schuijers & Clevers (2012) EMBO J. 31, 2685.
Rizk & Barker (2012) WIREs Syst Biol Med. 4, 475.

# *In Vivo* Duodenal Micronucleus Assay (90-day Study)

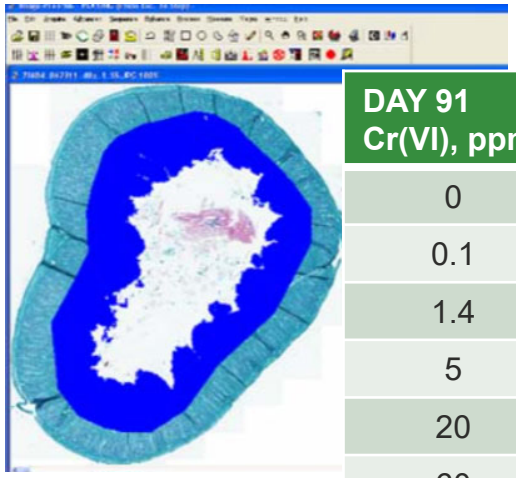
## Intact Crypts



| 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



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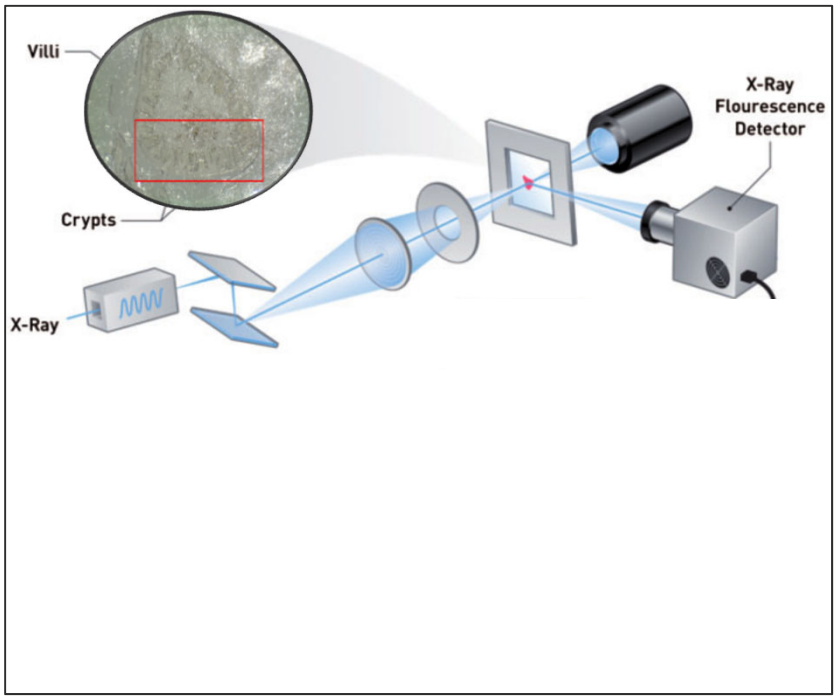
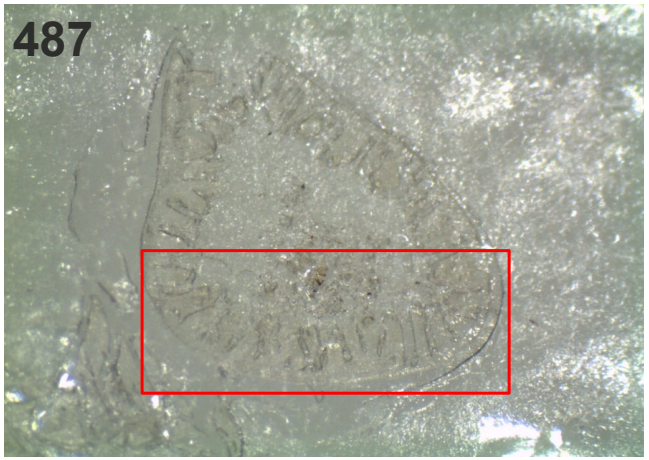
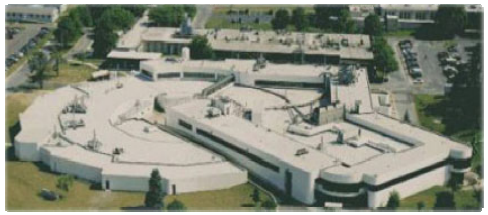
# Synchrotron Based X-ray Fluorescence (XRF) Microscopy



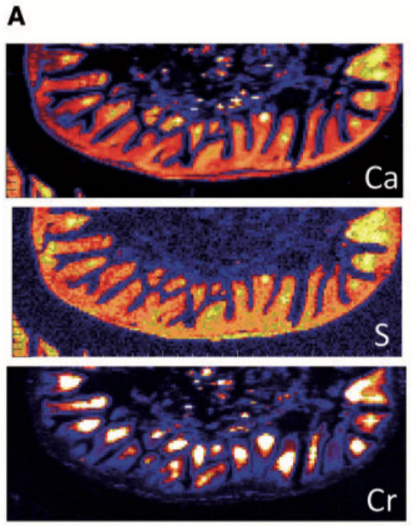




Synchrotron Light Source:



Brookhaven National Laboratory (Long Island, NY)

# XRF Maps of Cr, Ca, and S in Duodenum (90 Days of Exposure)




These findings would seem to preclude mutagenic MOA

Source:
Thompson et al. (2015) Tox Sci 143, 16.

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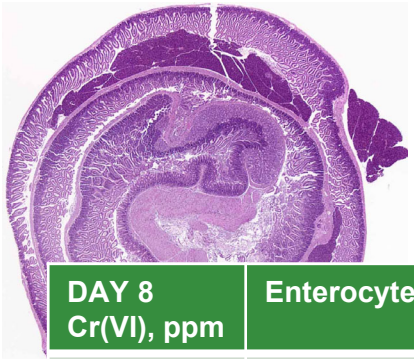
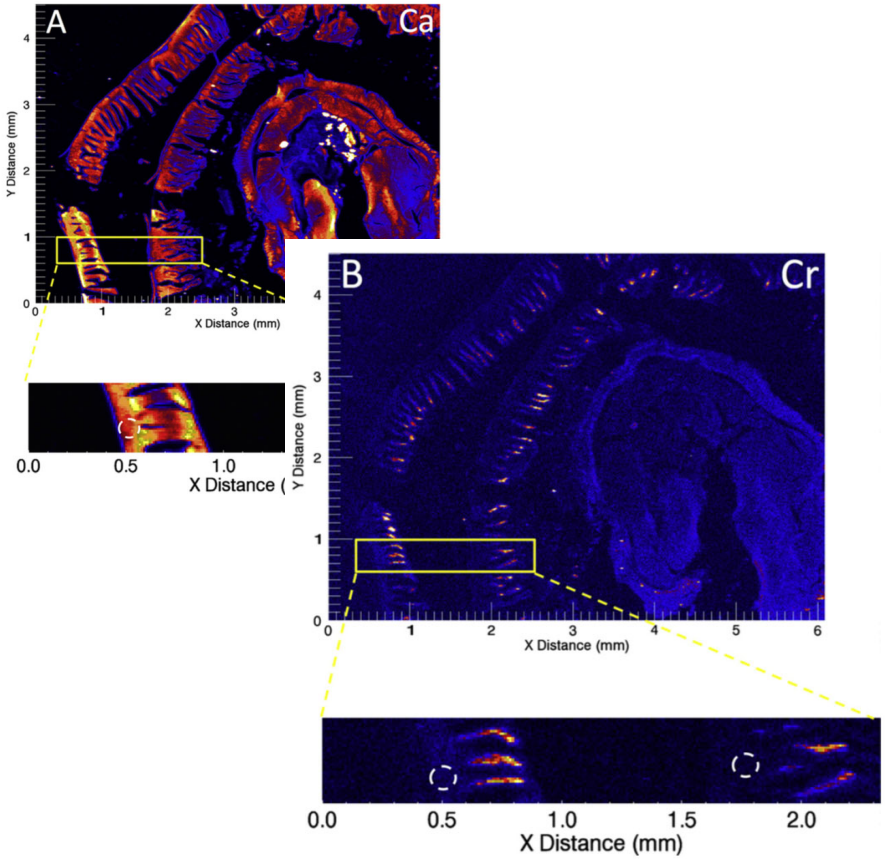
# *In Vivo* Duodenal Micronucleus Assay (7-day study)

MN Study ('Swiss Roll')



| DAY 8 Cr(VI), ppm | Enterocytes | Crypts |
|---|---|---|
| 0 | 6694 | 171 |
| 1.4 | 3159 | 77 |
| 21 | 3946 | 76 |
| 180 | 5161 | 77 |
| Cyclophos. | 3447 | 87 |

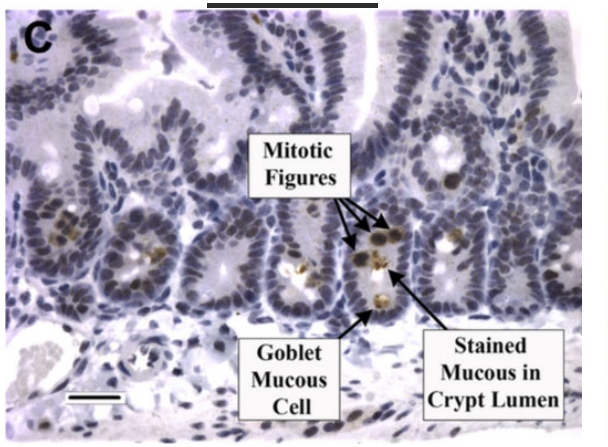
Source: Thompson et al. (2015) Mut Res 789-90, 61.




Note: bolded values are statistically significant

# γ-H2AX Immunostaining in 7-day MN Study (Swiss Roll Sections)

**Control**




γ-H2AX staining provides an additional approach for finding aberrant nuclei.

Source: Thompson et al. (2015) Mut Res 789-90, 61.



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# *In Vivo* Mutation Analysis: K-ras Codon 12 Mutations (90-day Exposure)

- 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

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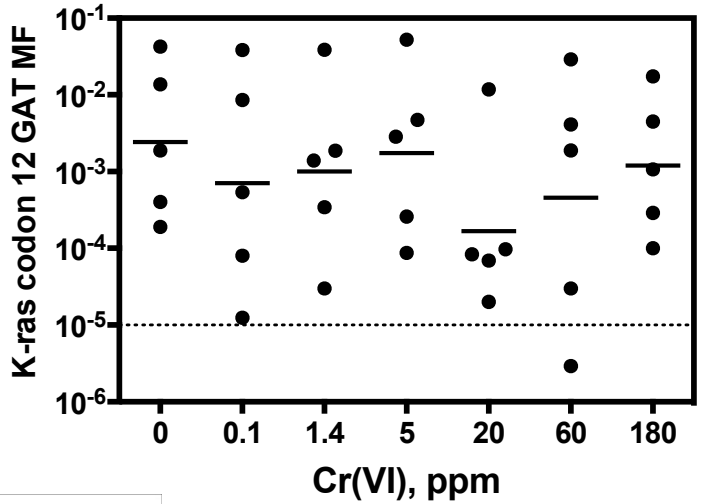
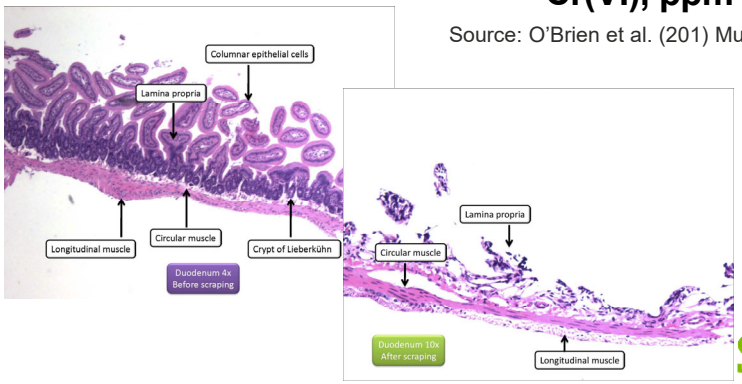
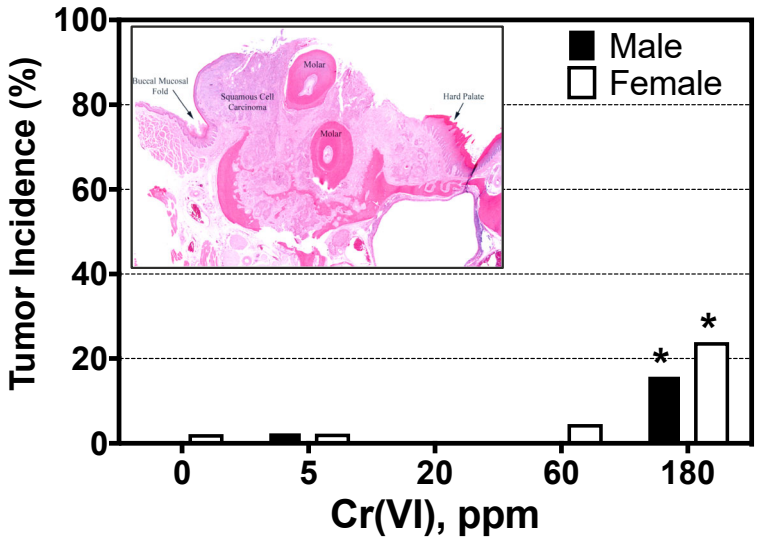


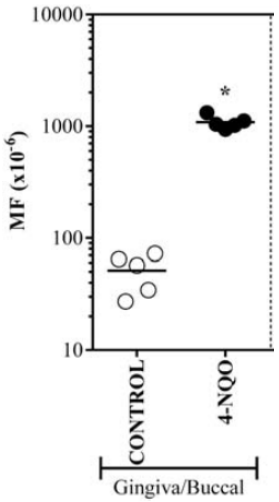

Source: O'Brien et al. (201) Mut Res 754, 15-21.




Strategies

# TGR Mutation Assay in Oral Mucosa of Big Blue® TgF344 Rats



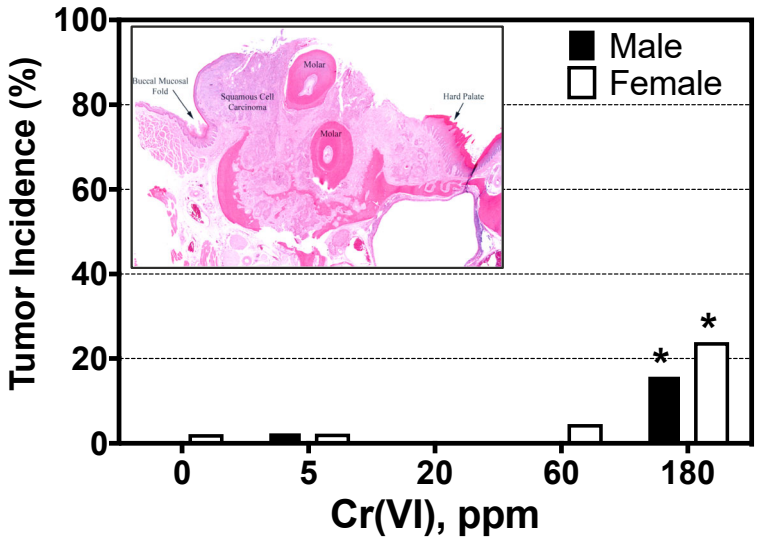



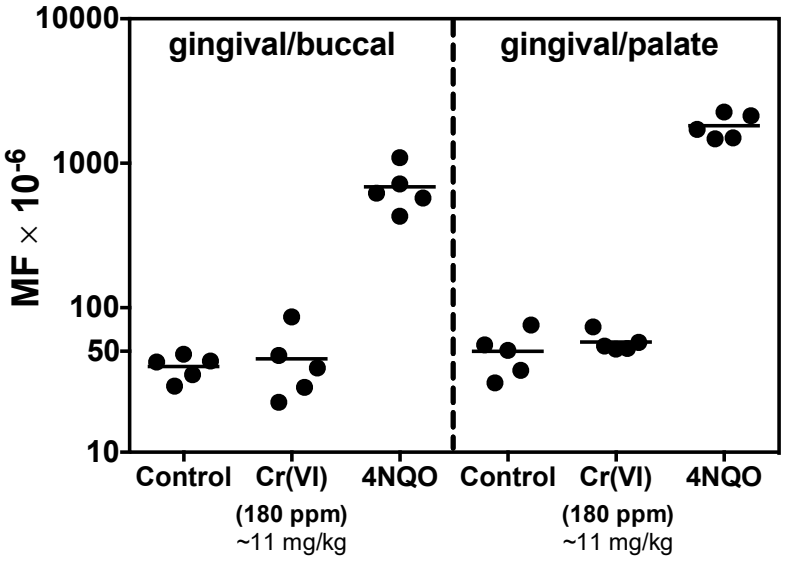

Source: Young et al. 2015 EMM 56, 629-636.



ToxStrategies

# TGR Mutation Assay in Oral Mucosa of Big Blue® TgF344 Rats




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



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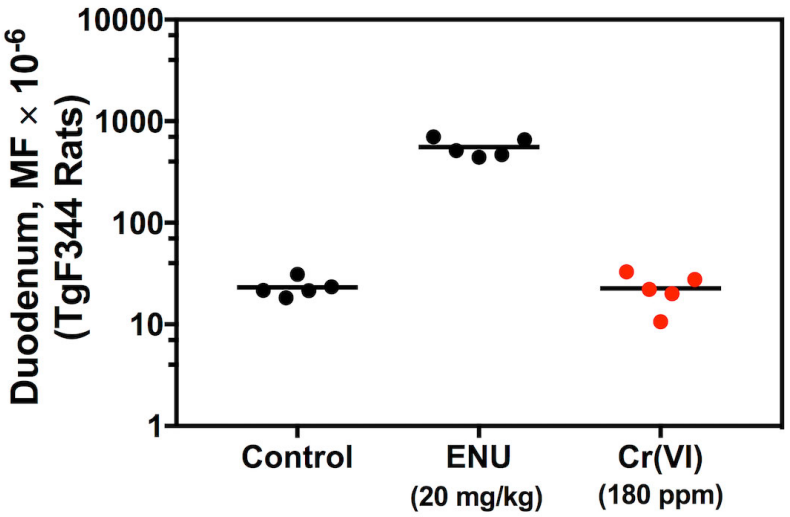
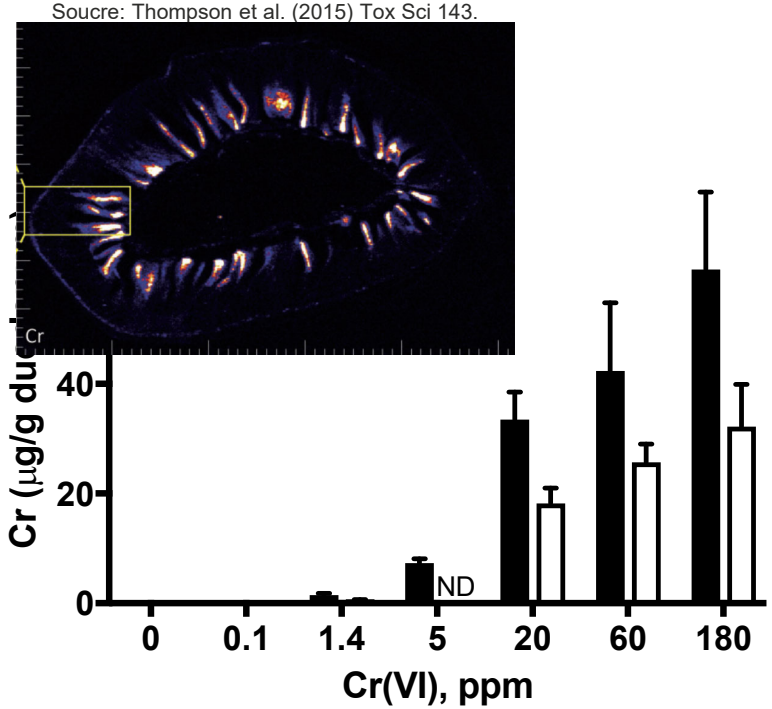
# TGR Mutation Assay in Duodenum of Big Blue® TgF344 Rats




Source: Thompson et al. 2017 TAP 330:48-52.

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



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# TGR Mutation Assay in Small Intestine of *gpt* Delta Mice


doi: 10.14252/foodsafetyfscj.2018014
Food Safety 2019; Vol. 0, No. 0, **–**

FOOD SAFETY

Published online in advance by J-STAGE

Original Article

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

Yasunobu Aoki[1], Michiyo Matsumoto[1], Michi Matsumoto[1], Kenichi Masumura[2], Takehiko Nohmi[2]

[1]National Institute for Environmental Studies, Center for Health and Environmental Risk Research, Tsukuba, Japan
[2]National Institute of Health Sciences, Division of Genetics and Mutagenesis, Kawasaki, Japan

The *in vivo* mutagenicity of hexavalent chromium in the small intestine, the target organ of tumorgenicity, was examined by means of a transgenic mouse gene mutation assay. Sodium dichromate dihydrate was administered orally in drinking water to male *gpt* delta mice at a dose of 85.7 or 257.4 mg/L for 28 days or at a dose of 8.6, 28.6 or 85.7 mg/L for 90 days. No significant increase in *gpt* mutant frequency relative to that in control mice was observed in the small intestine in either the 28- or 90-day study, whereas 28-day oral administration of potassium bromate, a positive control substance, increased mutant frequency.

**Key words:** genotoxicity, hexavalent chromium, *in vivo* mutagenesis, small intestine, transgenic rodent gene mutation assay, tumor

### Introduction

Hexavalent chromium compounds are categorized as Group I human carcinogens by WHO/IARC[1,2]. Exposure to hexavalent chromium has been shown in epidemiological studies to increase the risk of lung cancer[3], while there is little evidence of an association between hexavalent chromium exposure and the incidence of cancer in gastrointestinal organs such as the stomach. Experimental animal studies conducted by the National Toxicology Program have shown that exposure to the hexavalent chromium compound sodium dichromate via drinking water for 2 years increases the incidence of tumors of the oral mucosa or tongue in rats and of the small intestine in mice[4]. Therefore, the possibility of hexavalent chromium in drinking water to cause cancer in humans must be assessed.

Hexavalent chromium compounds are known to generate reactive oxygen species (ROS), which form oxidative adducts with DNA and proteins, resulting in activation of adverse outcome pathways such as genotoxicity and cytotoxicity[5]. However, the mechanism and activating pathways contributing to the carcinogenicity of hexavalent chromium in rodents have not been studied. Hexavalent chromium compounds show mostly positive results both in Ames tests and in *in vitro* genotoxicity assays using cultured mammalian cells[6,7]. In *in vivo* genotoxicity tests in rodents, hexavalent chromium compounds show negative results for micronucleus formation when administered via drinking water, whereas they show positive results in several *in vivo* tests after the gavage administration or intraperitoneal injection[6,7]. Therefore, the *in vivo* mutagenicity of hexavalent chromium compounds in a target organ is necessary to be evaluated prior to assess the cancer risk posed by hexavalent chromium. In present study, we analyzed changes in mutant frequencies in *gpt* delta mice

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The contents of this article reflect solely the view of the author(s).
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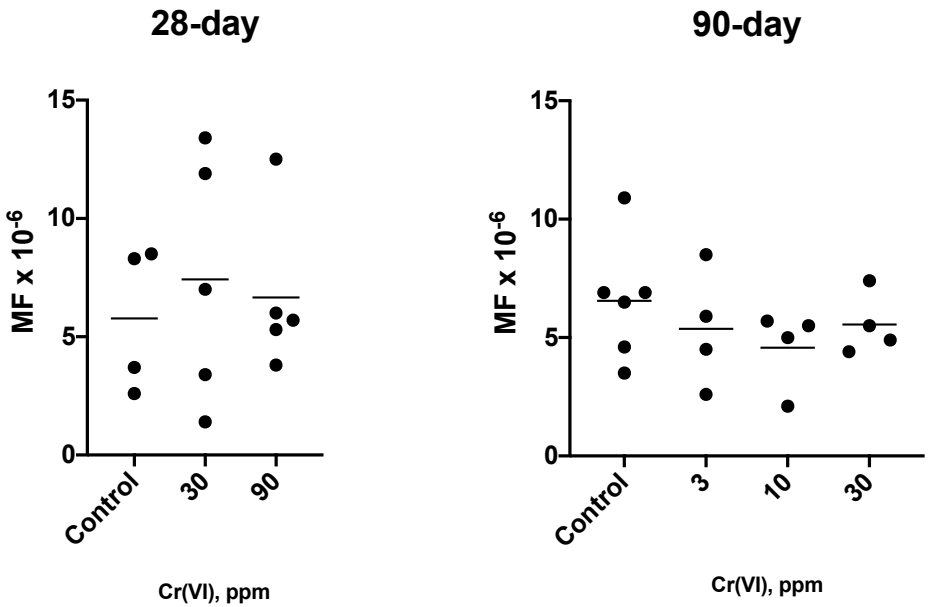




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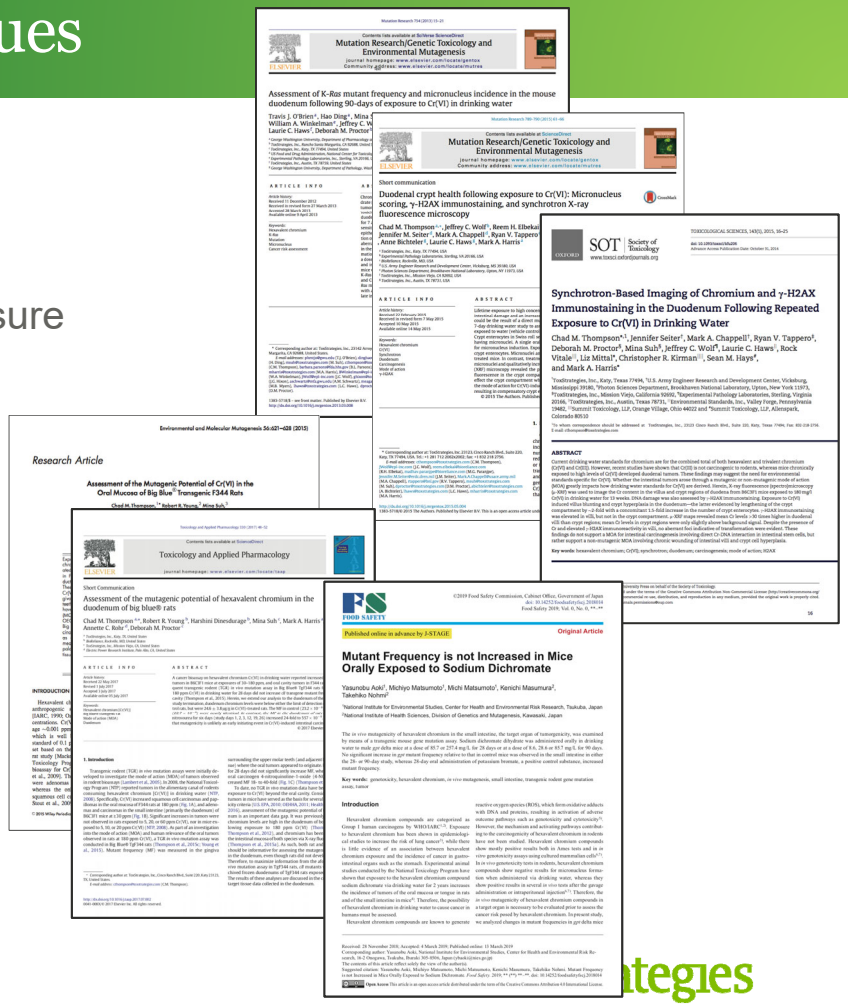
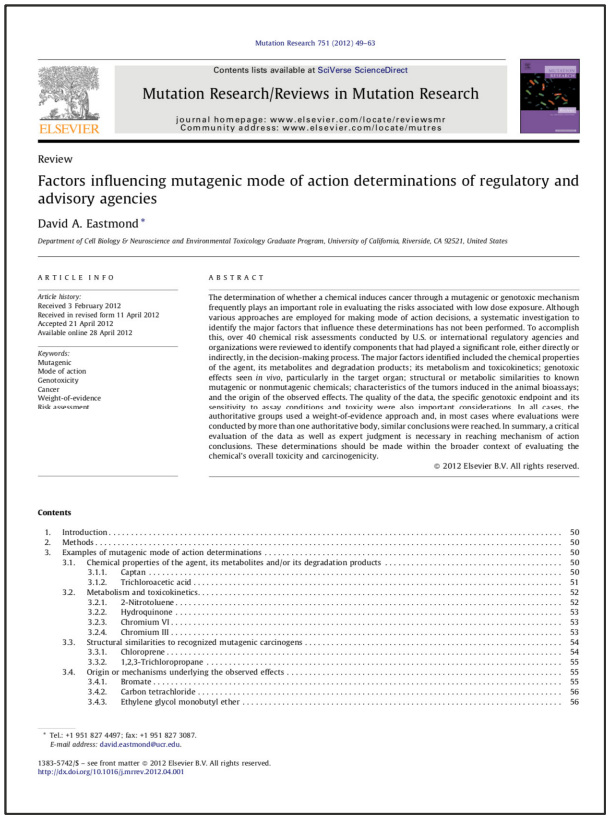
# *In Vivo* Genotoxicity in Target Tissues

- Duodenal MN assays
  - Neg after 7 and 90 days of exposure
- Duodenal γ-H2AX immunostaining
  - No diff from controls at 7 and 90 days of exposure
- *kras* codon 12 GAT MF in duodenum
  - Neg after 90 days of exposure
- XRF microscopy
  - Cr detected in villi (not crypt)
- Duodenal TGR assays
  - Neg in Big Blue rats after 28 days of exposure
  - Neg in *gpt* delta mice after 28 & 90 days exposure
- Oral mucosa mutation assay
  - Neg in Big Blue rats after 28 days of exposure
- Blood MN assays
  - most are neg.



# Factors for Assessing Mode of Action (MOA)



Mutation Research 751 (2012) 49–63

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Mutation Research/Reviews in Mutation Research

journal homepage: www.elsevier.com/locate/reviewsmr
Community address: www.elsevier.com/locate/mutres

Review

Factors influencing mutagenic mode of action determinations of regulatory and advisory agencies

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*Department of Cell Biology & Neuroscience and Environmental Toxicology Graduate Program, University of California, Riverside, CA 92521, United States*

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Cancer
Weight-of-evidence
Risk assessment

ABSTRACT

The determination of whether a chemical induces cancer through a mutagenic or genotoxic mechanism frequently plays an important role in evaluating the risks associated with low dose exposure. Although various approaches are employed for making mode of action decisions, a systematic investigation to identify the major factors that influence these determinations has not been performed. To accomplish this, over 40 chemical risk assessments conducted by U.S. or international regulatory agencies and organizations were reviewed to identify components that had played a significant role, either directly or indirectly, in the decision-making process. The major factors identified included the chemical properties of the agent, its metabolites and degradation products; its metabolism and toxicokinetics; genotoxic effects seen *in vivo*, particularly in the target organ; structural or metabolic similarities to known mutagenic or nonmutagenic chemicals; characteristics of the tumors induced in the animal bioassays; and the origin of the observed effects. The quality of the data, the specific genotoxic endpoint and its sensitivity to assay conditions and toxicity were also important considerations. In all cases, the authoritative groups used a weight-of-evidence approach and, in most cases where evaluations were conducted by more than one authoritative body, similar conclusions were reached. In summary, a critical evaluation of the data as well as expert judgment is necessary in reaching mechanism of action conclusions. These determinations should be made within the broader context of evaluating the chemical's overall toxicity and carcinogenicity.



**Contents**



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http://dx.doi.org/10.1016/j.mrrev.2012.04.001

| Factors Influencing MOA Determinations |
|---|
| Chemical properties |
| Toxicokinetics |
| Structural similarities to other carcinogens |
| Nature of the tumors |
| Mutational spectrum |
| Origin of mechanisms |
| Understanding assays |
| Quality, quantity, & reproducibility |
| ✓ In vivo genotoxicity (especially in target organs) |
| Evidence for an alternative MOA |



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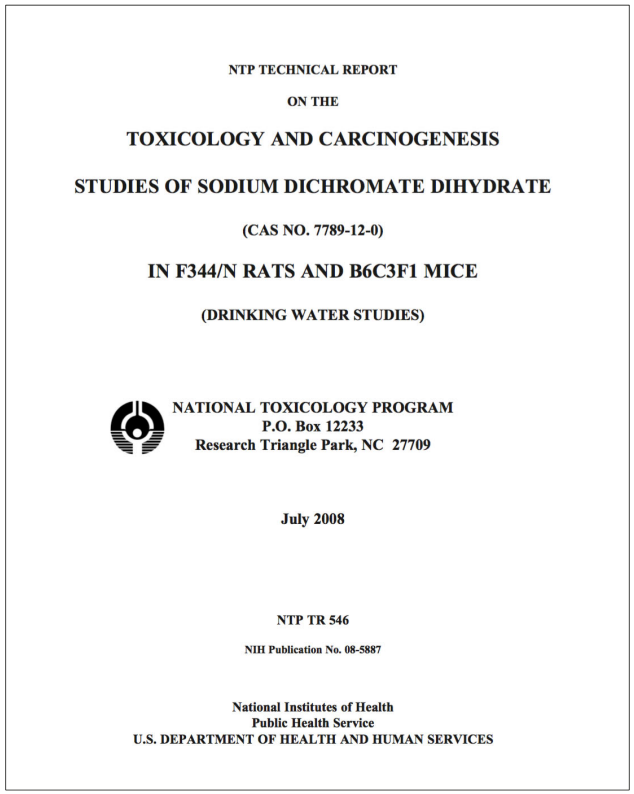
# Early Suggestions of Nonlinear Mechanisms



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

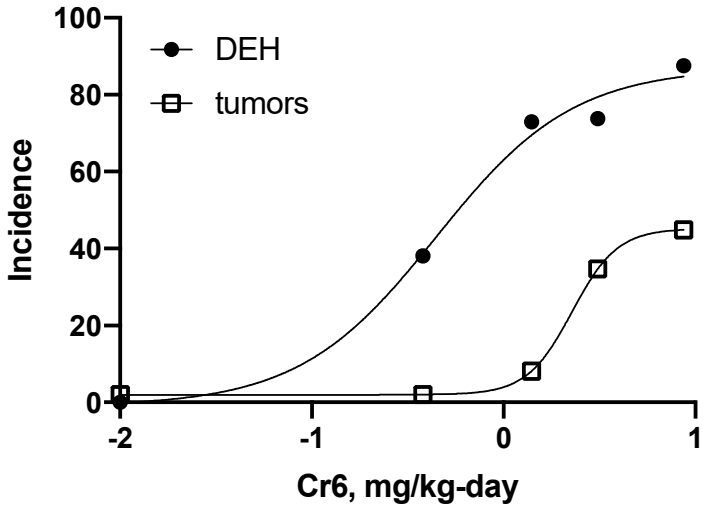
July 2008

NTP TR 546

NIH Publication No. 08-5887

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

- Diffuse epithelial hyperplasia (DEH) was observed in the duodenum of mice (but not rats) in the 13-wk bioassay
- DEH was observed in the duodenum of mice (but not rats) in the 2-year bioassay
- NTP (2008) study authors characterized DEH as secondary to mucosal injury in both 13-wk and 2-yr studies
- Duodenal tumors were only observed in mice

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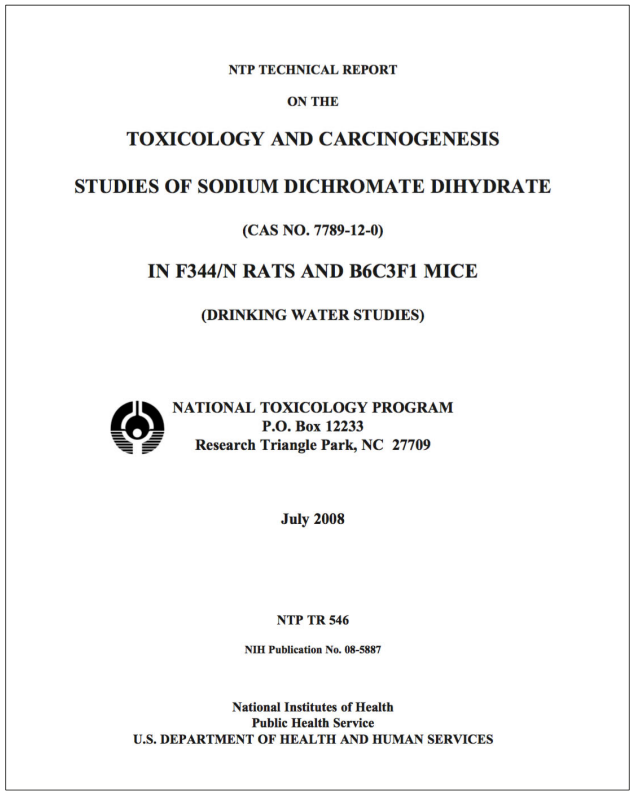
# Precedent for Cytotoxic-Regenerative Hyperplasia MOA for SI Tumors



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- 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 that do not cause cytotoxicity and regenerative cell hyperplasia"



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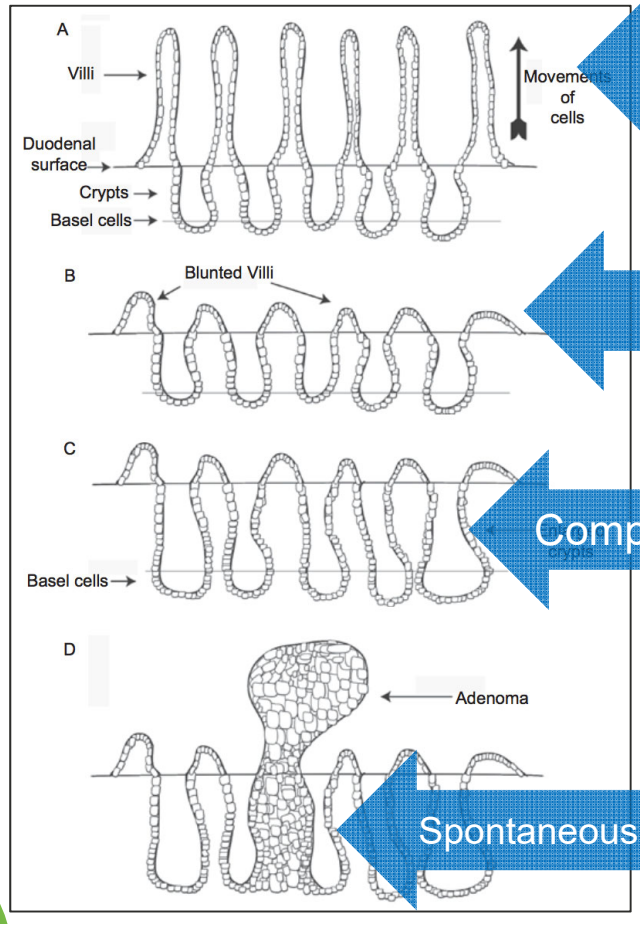
# Proposed MOA For Captan/Folpet




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

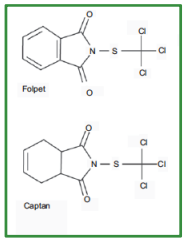
Chemical absorption at villi

Toxicity to villous enterocytes

Compensatory crypt hyperplasia

Spontaneous mutation leads to tumorigenesis

actually occur concomitantly

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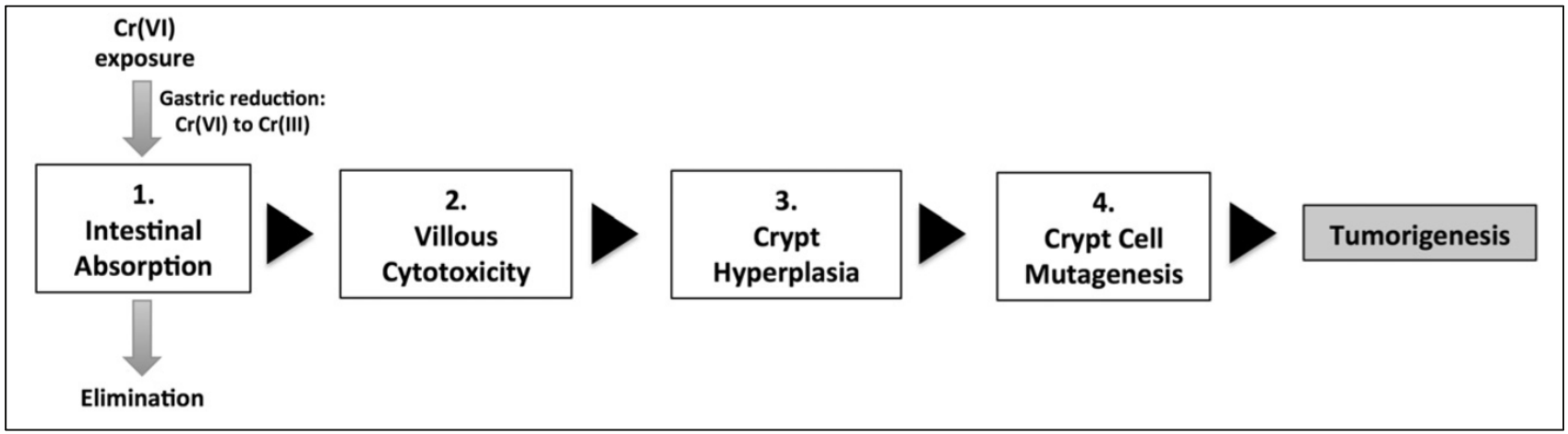
# Proposed Non-mutagenic MOA for Cr(VI)-Induced SI Tumors




Thompson et al. (2013) Crit Rev. Toxicol. 43, 244



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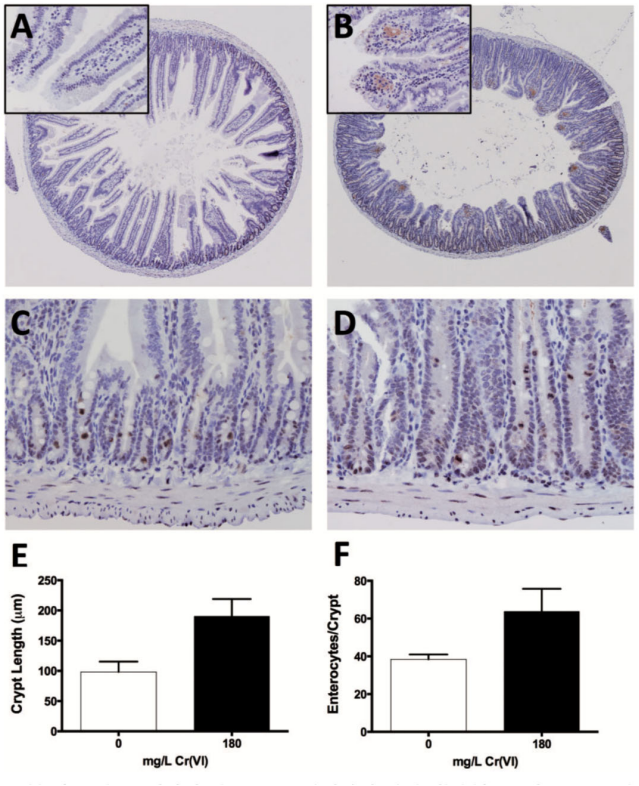
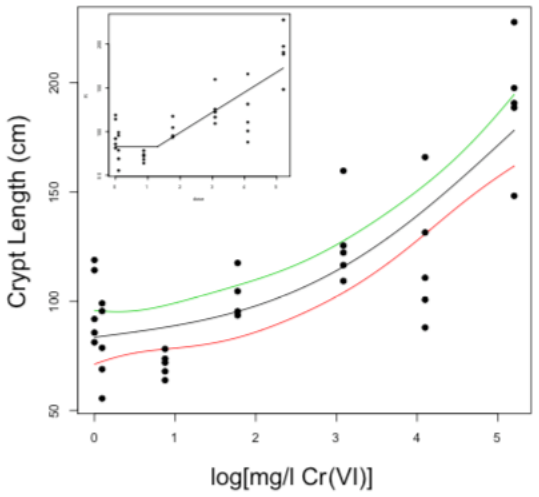
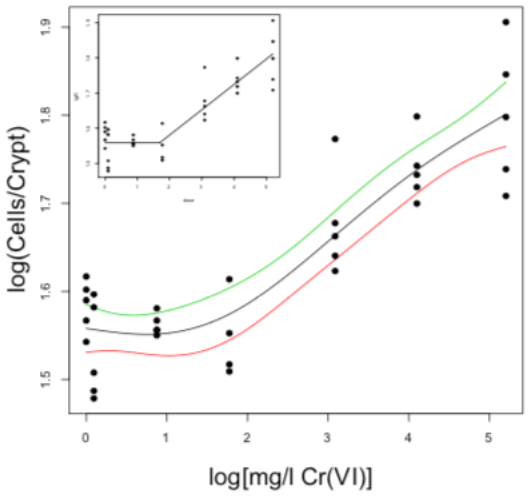
# Evidence of Mucosal Damage and Hyperplasia




Source: Thompson et al. 2015 Tox. Sci 143:16-25.



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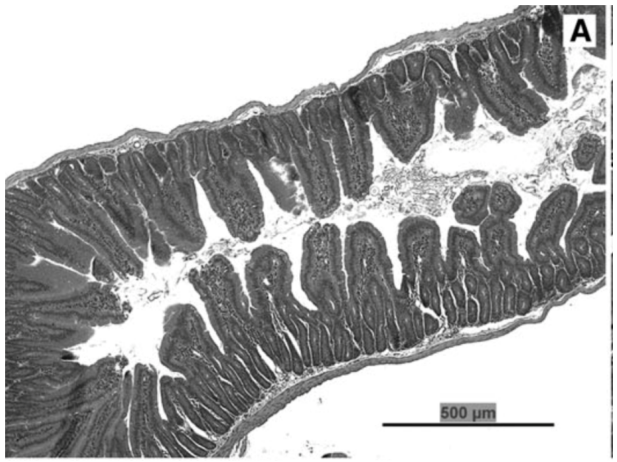
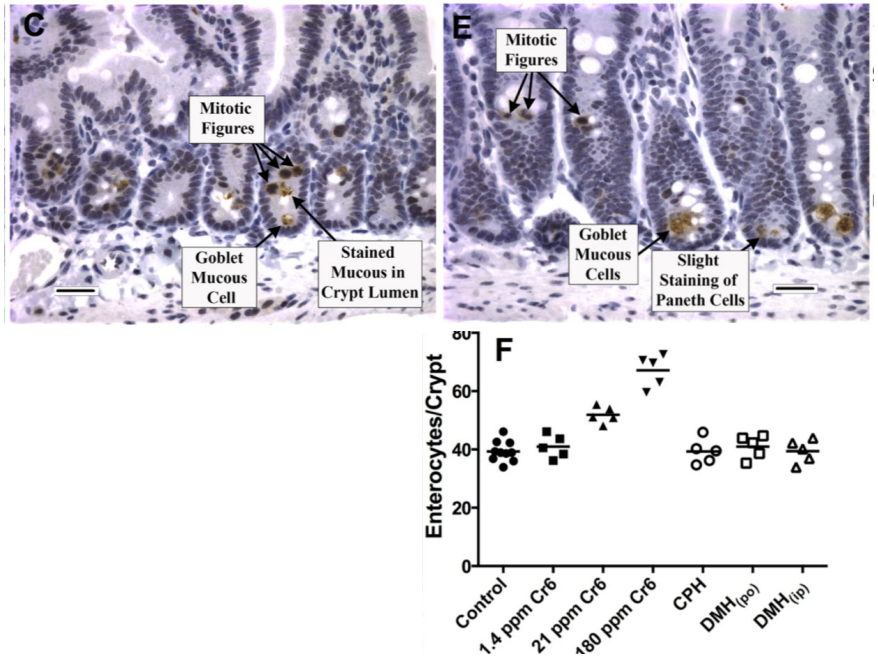
# Evidence of Mucosal Damage and Hyperplasia After 1 Wk Expsoure




Source: Thompson et al. (2011) Tox Sci 123: 58-70.




Source: Thompson et al. (2015) Mut Res 789-90, 61.



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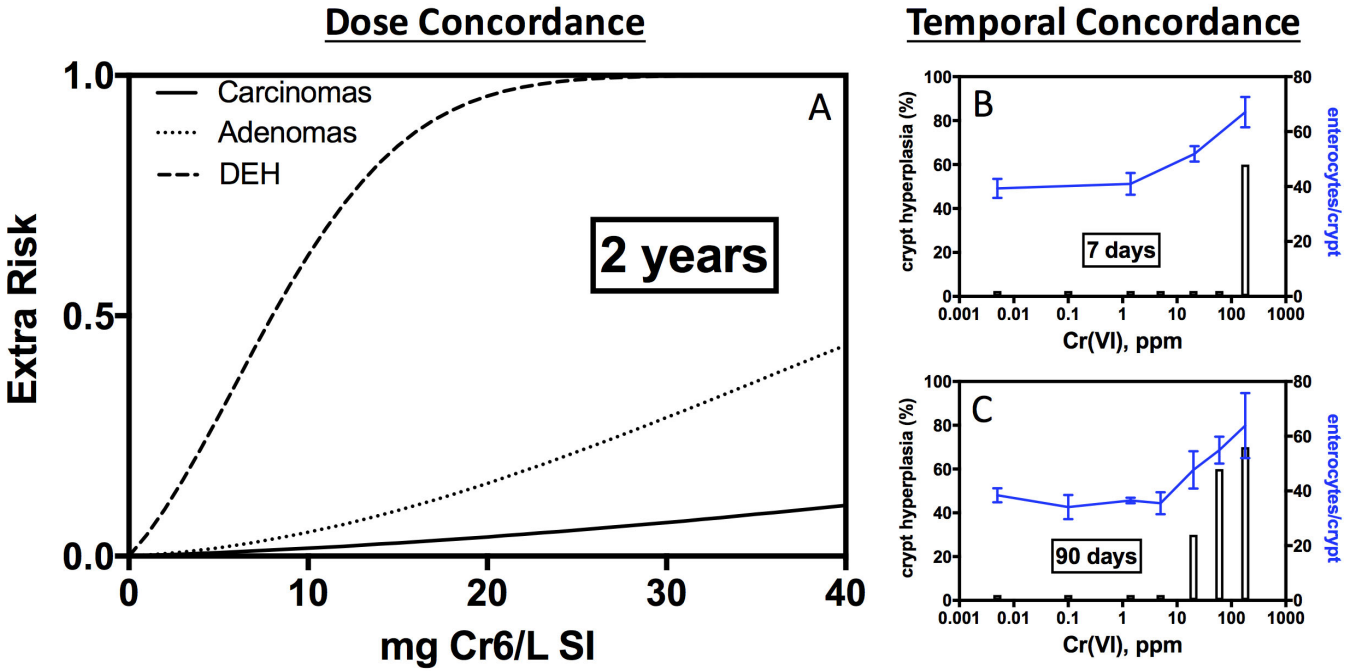
# Dose & Temporal Concordance for Hyperplasia



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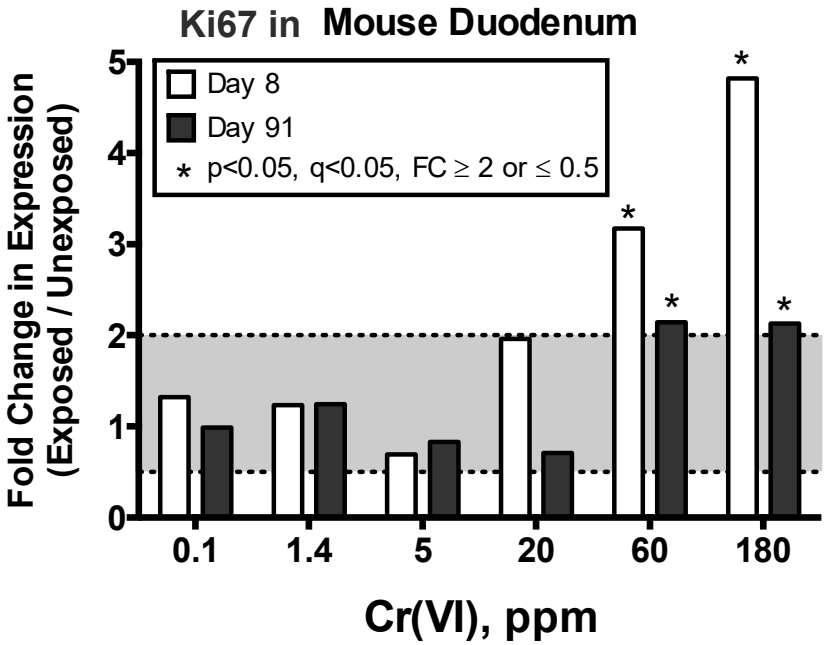
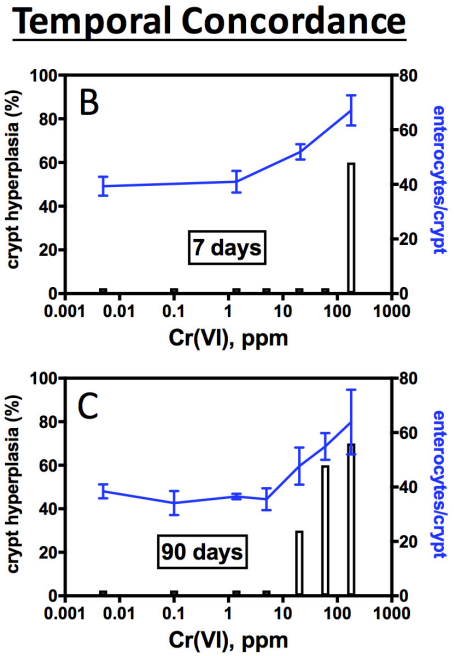
# Dose & Temporal Concordance for Hyperplasia



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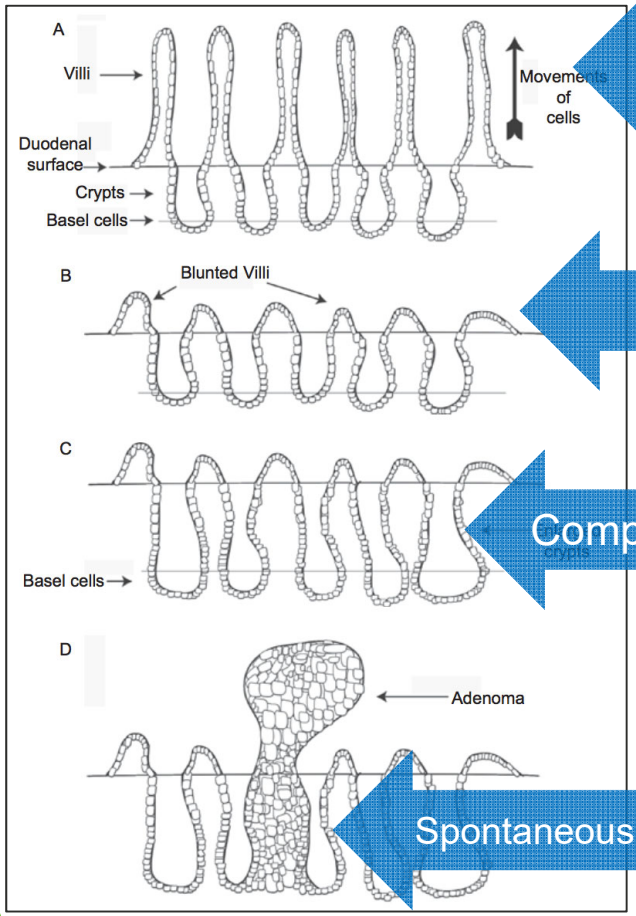
# MOA is Similar to that Proposed for Captan and Folpet

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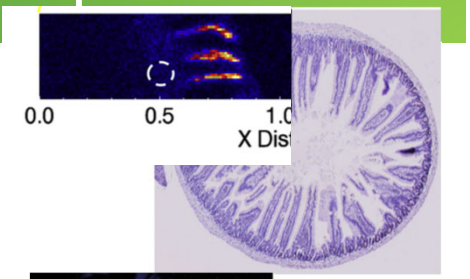
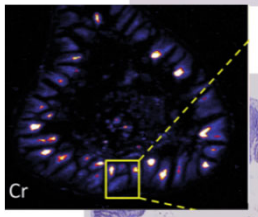
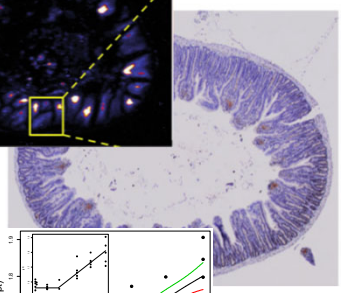
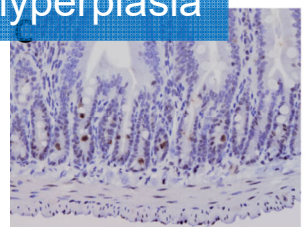
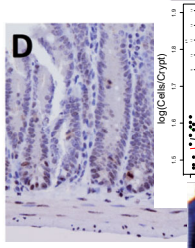
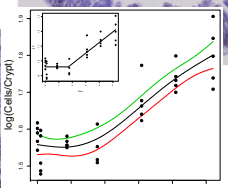


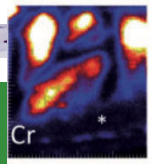





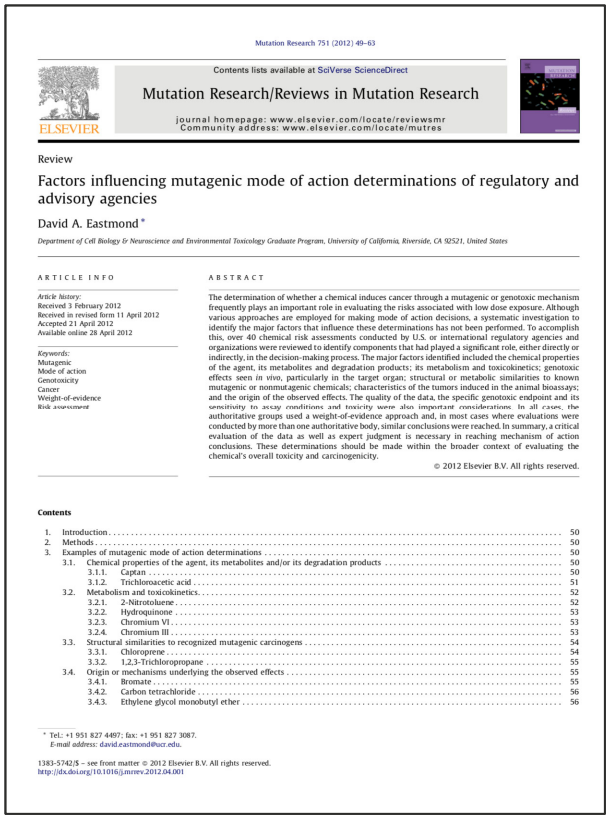



No *in vivo* genotox & no Cr in crypt.

# Factors for Assessing Mode of Action (MOA)



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Review

Factors influencing mutagenic mode of action determinations of regulatory and advisory agencies

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*Department of Cell Biology & Neuroscience and Environmental Toxicology Graduate Program, University of California, Riverside, CA 92521, United States*

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Risk assessment

ABSTRACT

The determination of whether a chemical induces cancer through a mutagenic or genotoxic mechanism frequently plays an important role in evaluating the risks associated with low dose exposure. Although various approaches are employed for making mode of action decisions, a systematic investigation to identify the major factors that influence these determinations has not been performed. To accomplish this, over 40 chemical risk assessments conducted by U.S. or international regulatory agencies and organizations were reviewed to identify components that had played a significant role, either directly or indirectly, in the decision-making process. The major factors identified included the chemical properties of the agent, its metabolites and degradation products; its metabolism and toxicokinetics; genotoxic effects seen *in vivo*, particularly in the target organ; structural or metabolic similarities to known mutagenic or nonmutagenic chemicals; characteristics of the tumors induced in the animal bioassays; and the origin of the observed effects. The quality of the data, the specific genotoxic endpoint and its sensitivity to assay conditions and toxicity were also important considerations. In all cases, the authoritative groups used a weight-of-evidence approach and, in most cases where evaluations were conducted by more than one authoritative body, similar conclusions were reached. In summary, a critical evaluation of the data as well as expert judgment is necessary in reaching mechanism of action conclusions. These determinations should be made within the broader context of evaluating the chemical's overall toxicity and carcinogenicity.



**Contents**



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http://dx.doi.org/10.1016/j.mrrev.2012.04.001

| Factors Influencing MOA Determinations |
|---|
| Chemical properties |
| Toxicokinetics |
| Structural similarities to other carcinogens |
| Nature of the tumors |
| Mutational spectrum |
| Origin of mechanisms |
| Understanding assays |
| Quality, quantity, & reproducibility |
| ✓ In vivo genotoxicity (especially in target organs) |
| ✓ Evidence for an alternative MOA |

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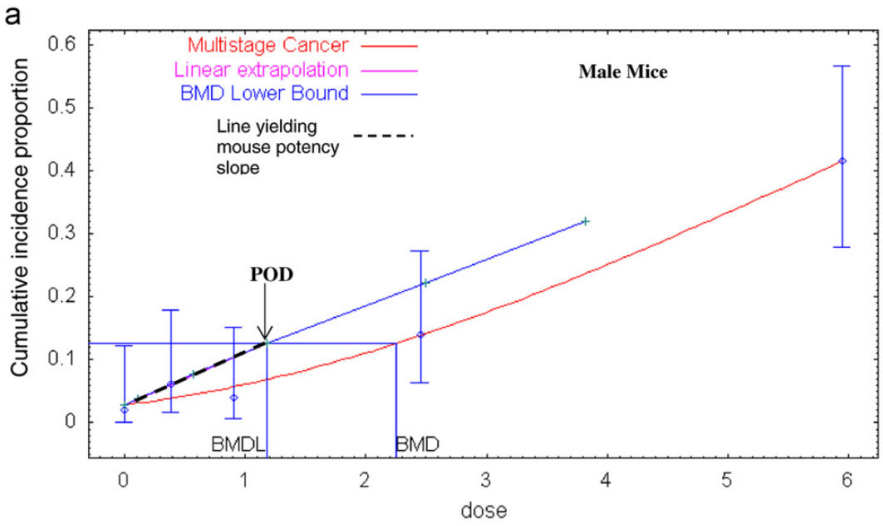
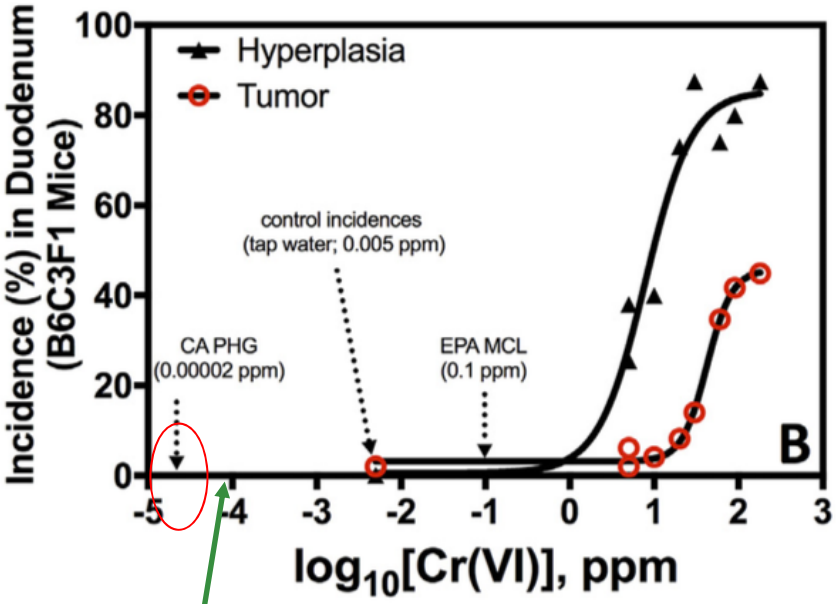
# Risk Assessment of Cr(VI) ca. 2010




Source: Stern 2010 Environ Res 110: 798-807.




Source: Thompson et al. 2017 TAP 330:48-52.

NJDEP
Ca. EPA
U.S. EPA DRAFT

**EPA & NJDEP** $10^{-6}$ risk water concentration (0.00007 ppm)



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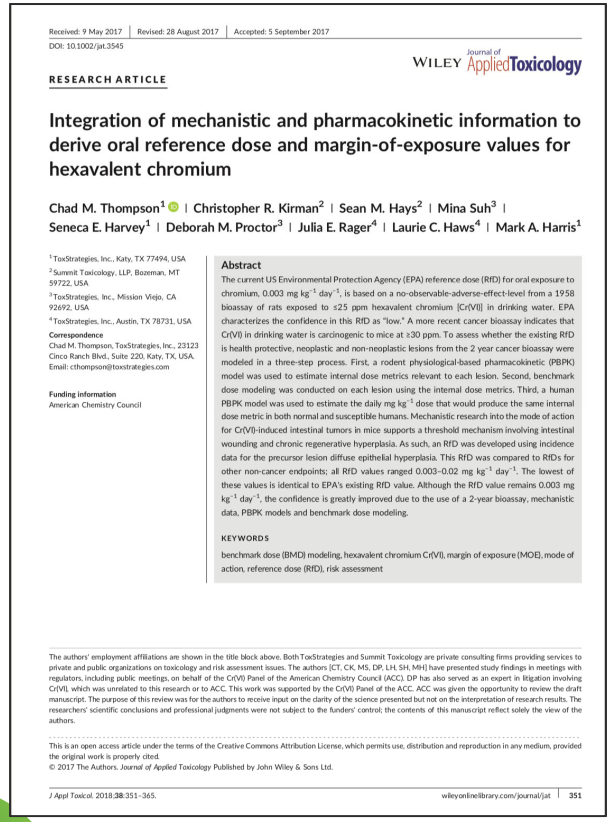
# Thompson et al. (2014, 2018)



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WILEY Journal of Applied Toxicology

RESEARCH ARTICLE

**Integration of mechanistic and pharmacokinetic information to derive oral reference dose and margin-of-exposure values for hexavalent chromium**

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**Funding information**
American Chemistry Council

**Abstract**

The current US Environmental Protection Agency (EPA) reference dose (RfD) for oral exposure to chromium, 0.003 mg $kg^{-1}$ $day^{-1}$, is based on a no-observable-adverse-effect-level from a 1958 bioassay of rats exposed to ≤25 ppm hexavalent chromium [Cr(VI)] in drinking water. EPA characterizes the confidence in this RfD as "low." A more recent cancer bioassay indicates that Cr(VI) in drinking water is carcinogenic to mice at ≥30 ppm. To assess whether the existing RfD is health protective, neoplastic and non-neoplastic lesions from the 2 year cancer bioassay were modeled in a three-step process. First, a rodent physiological-based pharmacokinetic (PBPK) model was used to estimate internal dose metrics relevant to each lesion. Second, benchmark dose modeling was conducted on each lesion using the internal dose metrics. Third, a human PBPK model was used to estimate the daily mg $kg^{-1}$ dose that would produce the same internal dose metric in both normal and susceptible humans. Mechanistic research into the mode of action for Cr(VI)-induced intestinal tumors in mice supports a threshold mechanism involving intestinal wounding and chronic regenerative hyperplasia. As such, an RfD was developed using incidence data for the precursor lesion diffuse epithelial hyperplasia. This RfD was compared to RfDs for other non-cancer endpoints; all RfD values ranged 0.003–0.02 mg $kg^{-1}$ $day^{-1}$. The lowest of these values is identical to EPA's existing RfD value. Although the RfD value remains 0.003 mg $kg^{-1}$ $day^{-1}$, the confidence is greatly improved due to the use of a 2-year bioassay, mechanistic data, PBPK models and benchmark dose modeling.

KEYWORDS

benchmark dose (BMD) modeling, hexavalent chromium Cr(VI), margin of exposure (MOE), mode of action, reference dose (RfD), risk assessment

The authors' employment affiliations are shown in the title block above. Both ToxStrategies and Summit Toxicology are private consulting firms providing services to private and public organizations on toxicology and risk assessment issues. The authors [CT, CK, MS, DP, LH, SH, MH] have presented study findings in meetings with regulators, including public meetings, on behalf of the Cr(VI) Panel of the American Chemistry Council (ACC). DP has also served as an expert in litigation involving Cr(VI), which was unrelated to this research or to ACC. This work was supported by the Cr(VI) Panel of the ACC. ACC was given the opportunity to review the draft manuscript. The purpose of this review was for the authors to receive input on the clarity of the science presented but not on the interpretation of research results. The researchers' scientific conclusions and professional judgments were not subject to the funders' control; the contents of this manuscript reflect solely the view of the authors.





- Rodent PBPK models were used to convert NTP 2-yr study doses into internal Cr(VI) dose metrics
- Dose metrics for the duodenum, jejunum, and ileum of both sexes were combined to create a single robust dose-response curve
- Tumor or hyperplasia incidence were modeled using EPA's BMDS software
- BMDL values based on internal doses were derived and a 3-fold interspecies UF applied to account for possible differences in pharmacodynamics
- Human PBPK model was used to predict human exposure that results in internal dose equivalent to BMDL
- Applied a 3-fold $EF_{HD}$ for potential differences in human pharmacodynamic differences and 2.4-fold $EF_{HK}$ based on difference in human gastric fluid pH ($50^{th}$ to $95^{th}$ percentile)
- RfD = 0.003 mg/kg; DWEL of 100 ppb

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# Pharmacokinetic Studies on Cr(VI)

- Proctor et al. (2012) measured reduction of Cr(VI) to Cr(III) in rodents and human gastric fluid
- Kirman et al. (2012) developed rodent PBPK model for Cr(VI)
- Kirman et al. (2013) developed human PBPK model for Cr(VI)
- Kirman et al. (2016) measured reduction of Cr(VI) in gastric fluid from fed and fasted humans
- Kirman et al. (2017) updated rodent and human PBPK models for Cr(VI)


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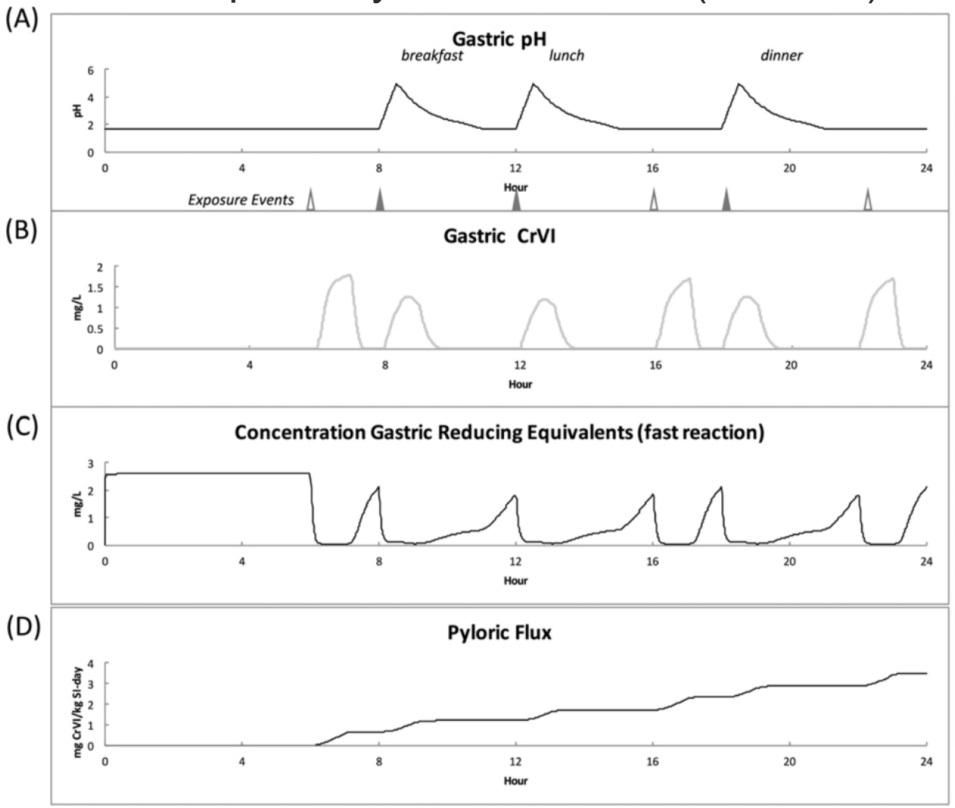
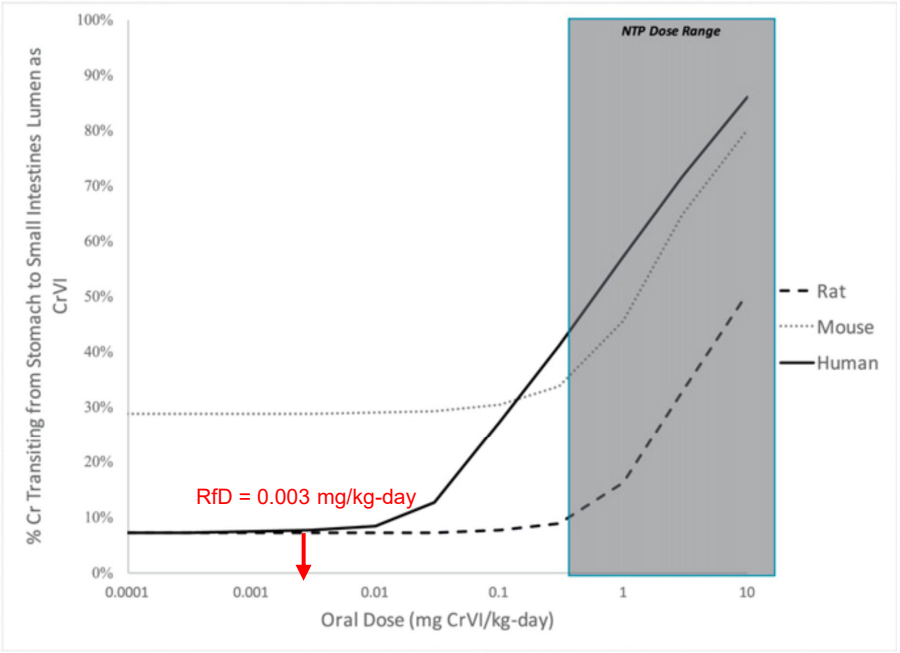
# Overview of Model and Results

## Example Daily Dose Estimate (Humans)

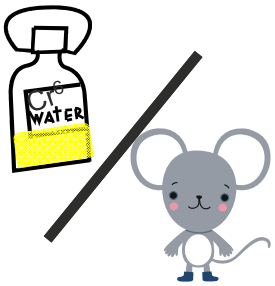



## Species Comparison of Cr(VI) Delivery to SI




Source: Kirman et al. (2017) TAP 325: 9-17.

# PBPK Model Used to Convert Applied Dose to Tissue Dose



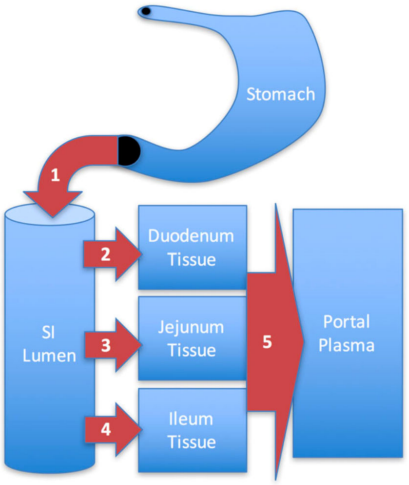
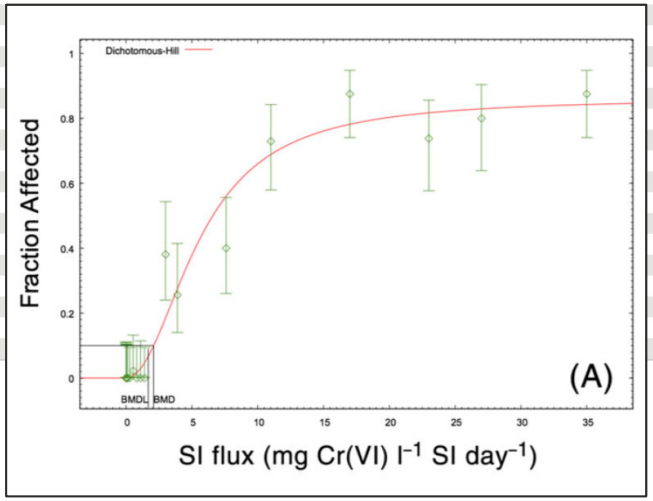




**TABLE 2** Dose–response data set for mouse intestinal effects using internal dose metrics

| Sex | Segment | SI sectional flux (mg kg$^{-1}$ SI day$^{-1}$) | N$^a$ (DEH) | DEH | N$^b$ (tumors) | Adenomas | Carcinomas | Combined |
|---|---|---|---|---|---|---|---|---|
| F | I | 1.4E-05 | 42 | 0 | 49 | 0 | 0 | 0 |
| M | I | 2.3E-05 | 40 | 0 | 49 | 0 | 0 | 0 |
| F | J | 1.7E-04 | 41 | 0 | 49 | 0 | 1 | 1 |
| M | J | 2.7E-04 | 41 | 0 | 49 | 0 | 0 | 0 |
| F | D | 3.0E-03 | 42 | 0 | 49 | 0 | 0 | 0 |
| M | D | 3.8E-03 | 39 | 0 | 49 | 1 | 0 | 1 |
| F | I | 2.8E-02 | 43 | 0 | 50 | 0 | 0 | 0 |
| M | I | 3.9E-02 | 42 | 0 | 49 | 1 | 0 | 1 |
| M | I | 1.2E-01 | 44 | 0 | 49 | 0 | 1 | 1 |
| F | I | 2.8E-01 | 47 | 0 | 49 | 0 | 0 | 0 |
| F | J | 2.9E-01 | 42 | 2 | 50 | 1 | 0 | 1 |
| M | J | 4.1E-01 | 42 | 0 | 49 | 0 | 2 | 2 |
| | I | 5.4E-01 | 45 | 1 | 50 | 0 | 0 | 0 |
| | I | | | | | | | |
| | | | | | | | | |
| | I | | | | | | | |
| | I | | | | | | | |
| F | J | | | | | | | |
| F | D | | | | | | | |
| M | J | | | | | | | |
| M | D | | | | | | | |
| F | J | | | | | | | |
| M | J | | | | | | | |
| M | D | | | | | | | |
| F | J | | | | | | | |
| F | D | | | | | | | |
| M | D | | | | | | | |
| F | D | | | | | | | |
| M | D | | | | | | | |
| F | D | | | | | | | |



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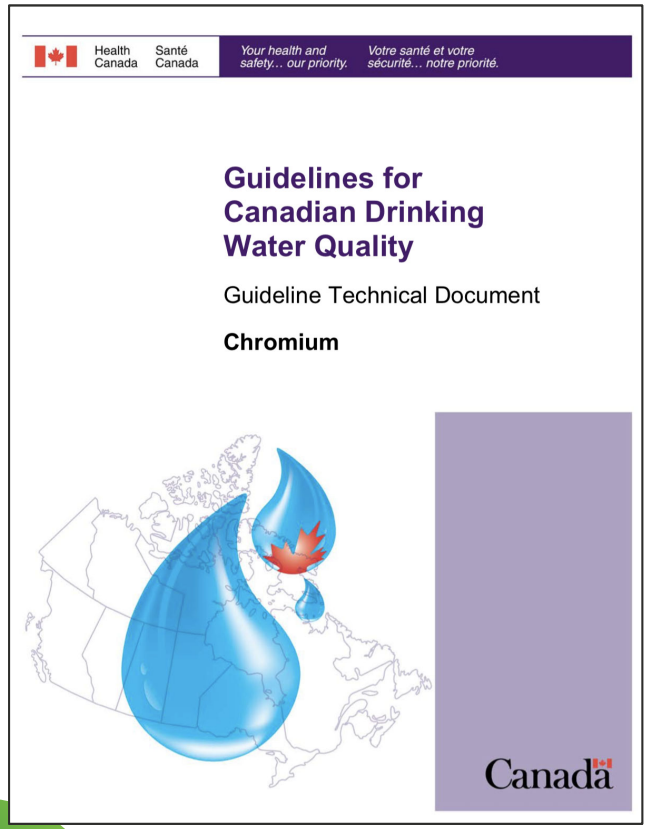
# Health Canada (2016)




- Similar approach as Thompson et al. (2014, 2018)
- Human PBPK model was used to predict human exposure that results in internal dose equivalent to BMDL (different assumptions than used in Thompsons et al.)
- Used HED for $BMDL_{01}$ for hyperplasia
- Applied 25-fold UF (2.5 interspecies; 10 intraspecies)
- TDI = 0.0022 mg/kg
- HBV = (TDI x 70 kg x 50% RSC)/1.5L = 50 ppb



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# TCEQ (2016)

Regulatory Toxicology and Pharmacology 73 (2015) 834–852

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Consideration of non-linear, non-threshold and threshold approaches for assessing the carcinogenicity of oral exposure to hexavalent chromium

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ABSTRACT

A non-linear approach, consiste
defensible for assessing the carci
the current paper builds upon pr
threshold approach as well as a
CrVI, and then utilizes available
supported approach. More speci
oped that adequately describes t
dose due to the sub-linear relat
tissue) across environmentally-
modeling was used to derive a
regenerative hyperplasia as a ke
RfD value shows remarkable agr
more scientifically-sophisticated,
et al., 2013b). The RfD approach
evidence of available MOA infor
MOA.

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1. Introduction

A significant amount of new research has been conducted over the past several years to generate data specifically to better inform the mode of action (MOA) analysis for hexavalent chromium-induced carcinogenesis due to oral exposure and to improve the extrapolation of rodent oral study results to humans (e.g., Thompson et al., 2011a, 2011b, 2012a, 2013a; Kirman et al., 2012, 2013; Proctor et al., 2012; Kopec et al., 2012a, 2012b; O'Brien et al., 2013; Suh et al., 2014; Thompson et al., 2015a, 2015c). Thorough evaluation of these research project data is essential to a better scientific understanding of the carcinogenic MOA operating in relevant rodent studies (e.g., NTP, 2008) and hexavalent chromium (CrVI) toxicokinetics following oral exposure, both of which are of particular importance considering the significant regulatory challenge of extrapolating high oral dose results from laboratory

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0273-2300/© 2015 The Author. Published by Elsevier Inc. This is an open access article und

Development Support Document
Final, September 23, 2016

**Hexavalent Chromium**
**Oral Reference Dose**

**CAS Registry Number: 18540-29-9**

Prepared by
Joseph T. Haney, Jr., M.S.
Toxicology Division

Office of the Executive Director
TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

- TCEQ value based on analysis published in Haney (2015)
- Modeled NTP hyperplasia data using 13-wk duodenal Cr levels from MOA research study (Kirman et al. 2012)
- Also modeled the relationship between duodenal levels and mg/kg bw dose
- Then converted the BMDL based on duodenal Cr levels to a mg/kg bw dose
- Applied 100-fold UF (10 interspecies; 10 intraspecies)
- RfD = 0.003 mg/kg; DWEL of 100 ppb ≅ MCL



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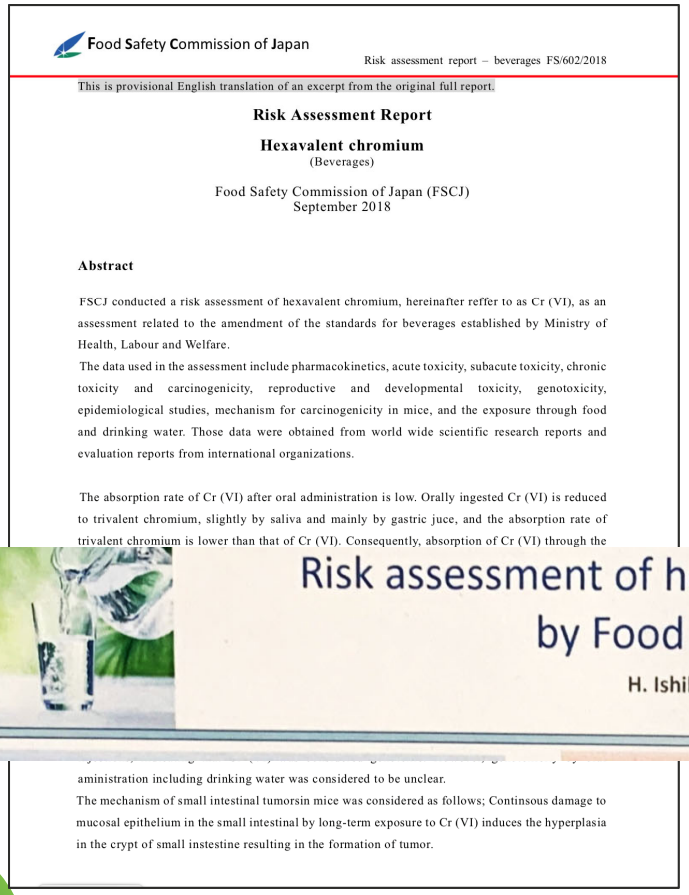
# Food Safety Commission of Japan (2018)



Food Safety Commission of Japan

Risk assessment report – beverages FS/602/2018

This is provisional English translation of an excerpt from the original full report.

**Risk Assessment Report**

**Hexavalent chromium**
(Beverages)

Food Safety Commission of Japan (FSCJ)
September 2018

**Abstract**

FSCJ conducted a risk assessment of hexavalent chromium, hereinafter reffer to as Cr (VI), as an assessment related to the amendment of the standards for beverages established by Ministry of Health, Labour and Welfare.

The data used in the assessment include pharmacokinetics, acute toxicity, subacute toxicity, chronic toxicity and carcinogenicity, reproductive and developmental toxicity, genotoxicity, epidemiological studies, mechanism for carcinogenicity in mice, and the exposure through food and drinking water. Those data were obtained from world wide scientific research reports and evaluation reports from international organizations.

The absorption rate of Cr (VI) after oral administration is low. Orally ingested Cr (VI) is reduced to trivalent chromium, slightly by saliva and mainly by gastric juce, and the absorption rate of trivalent chromium is lower than that of Cr (VI). Consequently, absorption of Cr (VI) through the

aministration including drinking water was considered to be unclear.

The mechanism of small intestinal tumorsin mice was considered as follows; Continsous damage to mucosal epithelium in the small intestinal by long-term exposure to Cr (VI) induces the hyperplasia in the crypt of small instestine resulting in the formation of tumor.

- Concluded that genotoxic mechanisms were unlikely to contribute to the tumors in rodents
  - Threshold can be established
- Modeled NTP hyperplasia data
- Applied 100-fold UF (10 interspecies; 10 intraspecies)
- TDI = 0.001 mg/kg

Risk assessment of hexavalent chromium (Cr(VI)) in drinking water by Food Safety Commission of Japan(FSCJ)

H. Ishibashi, M. Isozaki, N. Matsuzaki, M. Yoshida, H. Satoh

Food Safety Commission of Japan, Tokyo, Japan



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# Summary of Threshold Values Protective of Cancer

| Source | RfD or TDI (mg/kg-day) | Drinking Water (ppb) | Data Used |
|---|---|---|---|
| Thompson et al. (2018) | 0.003 | 100<br>(proposed keep MCL) | NTP data<br>PBPK models<br>MOA research |
| FSC of Japan (2018) | 0.001 | 30-60 | NTP data<br>No PK data<br>MOA research |
| Heath Canada (2016) | 0.0022 | 50<br>(same value as before) | NTP data<br>PBPK models<br>MOA research |
| Haney (2015), TCEQ (2016) | 0.003 | ≅ MCL | NTP data<br>PK data<br>MOA research |

*All values based on intestinal hyperplasia

# Update the Existing IRIS File for Chromium

**I.A.1. Oral RfD Summary**

| Critical Effect | Experimental Doses* | UF | MF | RfD |
|---|---|---|---|---|
| ~~None Reported~~ duodenal hyperplasia<br>~~Rat, 1-year drinking water study~~ 2-year bioassay<br>~~MacKenzie et al., 1958~~ NTP (2008) | NOAEL: 25 mg/L of chromium as $K_2CrO_4$ ~~2.5 mg/kg-day (adj.)~~ BMD modeling<br>~~LOAEL: None~~ | ~~300~~ | ~~3~~ | 3E-3 mg/kg-day remains the same |

**I.A.5. Confidence in the Oral RfD**

Study — ~~Low~~ High
Database — ~~Low~~ High
RfD — ~~Low~~ High

RfD is protective of cancer.



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# Summary

- Several toxicity criteria for Cr(VI) were developed immediately following the NTP (2008) bioassay
  - Assumed a mutagenic MOA
  - Used linear low-dose extrapolation approaches
- MOA research conducted from ~2010 to the present better inform the risk from oral exposure to Cr(VI)
  - Lack of genotoxicity in vivo (especially in target organs)
  - Strong evidence for a cytotoxicity-regenerative hyperplasia MOA
    - *Such a MOA has been accepted for SI cancer from captan and folpet*
  - Pharmacokinetic data suggest strong non-linearities in tissue dosimetry
- Recently developed toxicity criteria for Cr(VI) have utilized the MOA research
  - Concluded non-mutagenic MOA
  - Used non-linear (threshold) approaches for toxicity criteria