Report of the Chromium Science Advisory Board Review of *Research Framework for Evaluating the Potential Mode(s) of Action Underlying the Carcinogenicity of Hexavalent Chromium Following Exposure in Drinking Water*

Prepared by The Hamner Institute

Volume II

Expert Review Organized by Toxicology Excellence for Risk Assessment (*TERA*)
www.tera.org

July 28-29, 2009

The Friday Center
Chapel Hill, North Carolina

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Chromium Science Advisory Board Review of *Research Framework for Evaluating the Potential Mode(s) of Action Underlying the Carcinogenicity of Hexavalent Chromium Following Exposure in Drinking Water*

July 28-29, 2009

Panel Biographical Sketches and Conflict of Interest

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Panel Biographical Sketches and Conflict of Interest

An essential part of an independent expert review is the identification of conflicts of interest and biases that would disqualify a candidate, as well as identification and disclosure of situations which may appear to be a conflict or bias. *TERA* was selected by ToxStrategies to independently organize and conduct this expert panel review and was solely responsible for the selection of the panel. Prior to selecting *TERA* to conduct this expert review, the ToxStrategies reviewed information regarding *TERA* past and current work necessary to evaluate *'TERA's* independence. *TERA* has experience in risk assessment and toxicity of chromium from project work that has been done for a variety of public and private sponsors, but none of it is or was directly chromium mode of action research. *TERA* has not participated in the development or preparation of the research proposal that is the subject of this meeting. *TERA* has no other financial relationship with ToxStrategies and the sponsoring organizations and has no financial stake in the outcome of this review. As outlined in the contract between *TERA* and ToxStrategies, *TERA* independently selected the panel and organized this review. ToxStrategies had no influence on the selection of the panel or implementation of the process.

The purpose for evaluating conflict of interest is to ensure that the public and others can have confidence that the peer reviewers do not have financial or other interests that would interfere with their ability to carry out their duties objectively. *TERA* asked each promising candidate to report on his or her financial and other relationships with ToxStrategies, the Hamner Institute, and the member companies of the Aerospace Industry Association (AIA), which is providing funding for the research and the SAB.

The evaluation of real and perceived bias or conflict of interest was an important consideration in panel selection. *TERA* follows the U.S. National Academy of Sciences (NAS) guidance on selection of panel members to create panels that have a balance of scientific viewpoints on the issues to be discussed. As a result, the expert panels have a broad and diverse range of knowledge, experience, and perspective, including diversity of scientific expertise and affiliation. Panel members serve as *individuals*, representing their own personal scientific opinions. They do not serve as representatives of their companies, agencies, funding organizations, or other entities with which they are associated. Their opinions should not be construed to represent the opinions of their employers or those with whom they are affiliated.

Prior to selection, the candidates completed a questionnaire, which *TERA* used to determine whether their activities, financial holdings, or affiliations could pose a real or perceived conflict of interest or bias. The completed questionnaires were reviewed by *TERA* staff and discussed further with panel candidates as needed. (See www.tera.org/peer/COI.html for *TERA* conflict of interest and bias policy and procedures for panelist selection.)

TERA determined that the selected SAB members had no conflicts of interest and were able to objectively participate in this peer consultation. None of the panel members had a financial or other interest that would interfere with his or her abilities to objectively participate on the panel. None of the panel members was employed by ToxStrategies, the Hamner Institute, or the AIA

member companies. Nor did the panel members have any financial interests in these organizations or in the outcome of the review. None of the panel members were involved in the preparation of the research proposal.

A brief biographical sketch of each panel member is provided below. To promote transparency, a short statement describing situations which might appear to present a conflict of interest or bias are included, as appropriate.

Michael L. Dourson, PhD, DABT, FATS
Toxicology Excellence for Risk Assessment (TERA)

Dr. Michael Dourson is the President of Toxicology Excellence for Risk Assessment (*TERA*), a nonprofit corporation dedicated to the best use of toxicity data in risk assessment. Before founding *TERA* in 1995, Dr. Dourson held leadership roles in the U.S. Environmental Protection Agency as chair of EPA's Reference Dose (RfD) Work Group, as a charter member of the EPA's Risk Assessment Forum and as chief of the group that helped create the Integrated Risk Information System (IRIS). Dr. Dourson received his Ph.D. in Toxicology from the University of Cincinnati. Dr. Dourson has served on or chaired numerous expert panels, including peer review panels for EPA IRIS assessments, EPA's Risk Assessment Forum, *TERA* International Toxicity Estimates for Risk (*ITER*) independent peer reviews and consultations, FDA's Science Board Subcommittee on Toxicology, the NSF International's Health Advisory Board, and SOT's harmonization of cancer and non-cancer risk assessment. Dr. Dourson has organized numerous symposia on a variety of topics, including: risk communication; chromium; information resources for toxicology and environmental health; risk assessment of essential trace elements; risk characterization; EPA's IRIS; uncertainty in risk assessment techniques; statistical and dose response models in risk assessment; benchmark dose methodology; basics of risk assessment; improvements in quantitative noncancer risk assessment; and neurotoxicity risk assessment. Dr. Dourson is a Diplomate of the American Board of Toxicology and a Fellow of the Academy of Toxicological Sciences. In 2003, Dr. Dourson was awarded the Arnold J. Lehman award for major contributions that improve the scientific basis of risk assessment by the Society of Toxicology (SOT). He has been elected to multiple officer positions in the American Board of Toxicology, SOT, and the Society for Risk Analysis. He is also a media resource specialist in risk assessment for the SOT, member of the editorial board of three journals, and vice chair of the NSF International Health Advisory Board. Dr. Dourson has chaired dozens of expert panels reviewing risk assessments.

Dr. Dourson was selected for this panel for his expertise in dose-response assessment, metals toxicology, mode of action analysis, and familiarity with U.S. EPA's carcinogen risk assessment guidelines. In addition, Dr. Dourson has extensive experience effectively chairing panels of expert scientists in review of risk assessments.

Disclosure: Dr. Dourson is the President of *TERA*, which is under contract with ToxStrategies to independently organize and conduct this peer review. See discussion above. In 1995, Dr. Dourson testified in a legal case about the risk assessment approach used by U.S. EPA to develop the maximum contaminant level (MCL) for chromium. *TERA* has concluded that this is not a conflict of interest or a bias because Dr. Dourson's testimony was limited to explaining how existing U.S. EPA standards were derived, and he did not provide his professional opinion on the toxicity of chromium at concentrations under discussion.

Jeffery Fisher, PhD, FATS
University of Georgia

Dr. Fisher is a Professor in the Department of Environmental Health Science, College of Public Health at the University of Georgia and is Director of the Interdisciplinary Toxicology Program. Previously, he was Department Head and Professor in the Department of Environmental Health Science and the College of Agricultural and Environmental Sciences at the University of Georgia. Dr. Fisher's research interests are in the development and application of biologically based mathematical models to ascertain health risks from environmental and occupational chemical exposures. Dr. Fisher has a B.S. degree in biology (1973) from the University of Nebraska at Kearney, a M.S. degree in biology/ecology in (1979) from Wright State University, and a Ph.D. in Zoology/Toxicology (1987) from Miami University. Dr. Fisher has been a member of SOT since 1990. He served as Vice President (2002-2003) and President (2003-2004) of the Biological Modeling Specialty Section. He is an Associate Editor for Toxicological Sciences since (2008-present). He has supported SOT annual meetings by chairing poster sessions, and organizing and speaking at workshops and educational forums. He is a Fellow of the Academy of Toxicological Sciences (2006-present). Dr. Fisher is a member of the U.S. EPA's Science Advisory Board serving on the Exposure and Human Health Committee and is a member of the NRC/NAS AEGL subcommittee. He is currently on the editorial boards for the International Journal of Toxicology and the Journal of Toxicology and Environmental Health. Dr. Fisher's laboratory and computational research are funded through grants provided by the U.S. EPA, Center for Disease Control (CDC)/Agency for Toxic Substances and Disease Registry (ATSDR), Air Force Office Science Research, Department of Energy, and occasionally subcontracts with nonprofit organizations or trade groups.

Dr Fisher was selected for this panel for his expertise in the development of physiologically based pharmacokinetic models and his experience in serving on panels of expert scientists in review of risk assessments.

Disclosure: None.

David Gaylor, PhD, FATS
Gaylor and Associates

Dr. Gaylor is retired from the National Center for Toxicological Research (NCTR) of the Food and Drug Administration (FDA), where he was Director of the Division of Biometry and Risk Assessment. Currently, he is a consultant in the area of quantitative health risk assessment. Dr. Gaylor is also an Adjunct Professor of Biostatistics, University of Arkansas for Medical Sciences. He obtained a Ph.D. in Statistics from North Carolina State University in 1960 followed by employment with the Research Triangle Institute and the National Institute of Environmental Health Sciences. Dr. Gaylor's research has focused on the statistical design and analysis of toxicological experiments and the development of techniques for quantitative health risk assessment. He has published more than 180 journal articles, 25 book chapters, and made over 100 presentations at scientific conferences. Dr. Gaylor has served on more than 70 national and international committees on aspects of biometry, toxicology, and risk assessment for the FDA, U.S. EPA, CDC, World Health Organization, Health Canada, International Life Sciences Institute, and the National Research Council. He is a Fellow of the Academy of Toxicological Sciences, American Statistical Association, and Society for Risk Analysis. He currently is a member of the editorial board for Risk Analysis, Human and Ecological Risk Assessment, Regulatory Toxicology and Pharmacology, and Toxicology and Industrial Health.

Dr Gaylor was selected for this panel for his expertise in biostatistics, dose-response assessment, and for his experience in serving on panels of expert scientists in review of risk assessments.

Disclosure: Dr. Gaylor indicated that he is a past employee of General Electric, Co. and General Dynamics, Co. In addition, he has consulted in the past with 3M Corporation (2002-May 2009). All three companies are currently members of Aerospace Industry Association, which is the Sponsor of the review. *TERA* concluded that there is no conflict of interest or bias because none of the past work involved chromium and Dr. Gaylor has no current, ongoing financial relationship with these companies.

Kirk Kitchin, PhD, DABT
NHEERL, U.S. EPA

Dr. Kirk Kitchin is a Research Toxicologist in the Genetic and Cellular Toxicology Branch, Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development at the U.S. EPA in Research Triangle Park, NC. Dr. Kitchin received his BA in Chemistry (1972) and his PhD in Toxicology (1976) from the University of Rochester. His research interests include the design and conduct of mode of action studies and dose-response studies in experimental animals and/or other biological systems. His elaborate dose-response studies have included arsenic, 1,2-dimethylhydrazine, 1,2-dibromoethane and TCDD as study agents. These dose response studies generated data that can be used to select alternative risk assessment extrapolation methods below the dose range in which meaningful animal or human tumor observations are possible.

Dr. Kitchin has served as the coordinator of arsenic research for U.S. EPA's Environmental Carcinogenesis Division for many years. He was also a member of the team that prepared U.S. EPA's Research Plan for Arsenic in Drinking Water document (EPA/600/R-98/042) and served as a reviewer for U.S. EPA's Arsenic Health Assessment. Dr. Kitchin is a Diplomate of the American Board of Toxicology. He is a member of the Society of Toxicology where he has served as a Councilor, Vice-president, and President of the Metal Specialty Section, and he is Chairman of the
International Society for the Study of Xenobiotics' Committee on Regulatory Affairs. Dr Kitchin has served as member of the Editorial Board for the journals Toxicology and Toxicology Letters; he has numerous publications and invited presentations in the areas of mechanisms of carcinogenicity and arsenic carcinogenicity. Dr. Kitchin was selected for this panel for his expertise in metals carcinogenicity, cancer mode of action research, and use of genomics in risk assessment.

Disclosure: None.

Bette Meek, M.Sc.
University of Ottawa

Ms. Meek is currently the Associate Director of Chemical Risk Assessment with the McLaughlin Institute of the University of Ottawa on interchange from Health Canada, where she manages the Existing Substances Division in the Safe Environments Programme. Her responsibilities in this capacity are related to development and implementation of process and methodology for the assessment of the effects on human health of Existing Substances under the Canadian Environmental Protection Act, including setting priorities for assessment from among all 23,000 commercial chemicals used in Canada by September, 2006 (i.e., categorization). Ms. Meek received her M.Sc. in Toxicology with distinction from the University of Surrey in the UK in 1891. She is currently a PhD candidate at the University of Utrecht in the Netherlands. Ms. Meek has considerable experience in the development of methodology for and evaluation of health-related data on environmental contaminants, having also managed previously programmes within Health Canada on contaminants of drinking water and air. She acts as an advisor to several international organizations and has authored over 150 scientific publications in this area. Specific areas of experience include development of frameworks to increase transparency in the assessment of human relevance of animal modes of action, increasing incorporation of biological data in dose-response as a replacement for default, development of predictive exposure and hazard modelling and increasing efficiency in assessment through effective problem formulation and early and continuing peer engagement.

Ms. Meek was selected for this panel for her expertise in genotoxicity, dose-response assessment, and mode of action analysis in a regulatory context. In addition, Ms Meek has experience in serving on panels of expert scientists in review of toxicity assessments.

Disclosure: None.

Xianglin Shi, PhD
University of Kentucky

Dr. Shi is currently a Professor and the William A. Marquard Chair in Cancer Research, University of Kentucky,. Prior to this position he was an Expert Consultant in the Pathology and Physiology Research Branch for the National Institute for Occupational Safety and Health, in Morgantown, WV, for 4 years. Dr. Shi received his PhD in Chemistry from West Virginia University in 1988. Since then Dr. Shi has published over 300 articles and is involved in many professional societies. His current laboratory focuses on investigation on molecular mechanism of metal carcinogenesis, including studying metal-induced generation of reactive oxygen species, oxidative stress, activations of transcription factors, gene expression, cell migration and invasion, actin filament reorganization, transformation, tumorigenesis, DNA damage and repair, cell cycle regulation, and apoptosis. The long term goals of his research are to provide a fundamental understanding concerning the mechanism of carcinogenic actions of metal compounds; to fill a need for the mechanistic information of cancer risk assessment for metal exposure; to propose methods for early detection; and to develop intervention and prevention strategies, using electron spin resonance (ESR) spectrometer and general molecular biology techniques. Dr. Shi has contributed to the understanding of Cr(VI) carcinogenesis by establishing Cr(VI)-induced hydroxyl radical generation via Fenton and Haber Weiss reaction, identifying the cellular reduction of Cr(VI) by flavoenzymes, evaluating Cr(VI)-induced DNA damage through free radical reaction, and assessing Cr(VI) induced cell growth arrest, apoptosis, and activation of various signal pathways. Dr. Shi has organized numerous meetings and edited several journal special issues in the areas of metal carcinogenesis and oxidative stress. Dr. Shi's active research grants are all in the area of mechanisms of metals carcinogenesis from the National Institutes of Health.

Dr. Shi was selected for this panel due to his expertise in chromium mode of action research, particularly for his understanding of the role of oxidative stress and DNA damage in chromium toxicity. Dr. Shi also has expertise in genomics, proteomics, transcriptional analysis, and imaging.

Disclosure: None.

Patrick Winter, PhD
Washington University

Dr. Winter is currently a Research Assistant Professor in Medicine and Affiliate Assistant Professor of Biomedical Engineering at Washington University in St. Louis Missouri. Dr. Winter received a B.S. in Electrical Engineering from Washington University in 1994 and a Ph.D. in Biomedical Engineering from the University of Texas at Arlington in 2000. His research focuses on developing new magnetic resonance imaging (MRI) contrast agents and methods for molecular imaging in atherosclerosis and cancer. In particular, he has pioneered the use of targeted nanoparticles for molecular imaging and drug delivery applications. His primary areas of interest include imaging angiogenesis and anti-neovascular therapy in solid tumors, visualization of angiogenesis in atherosclerosis, detection of fibrin in atheromatous plaques and monitoring angiogenesis in peripheral vascular disease. He has also served as a consultant to pharmaceutical research organizations (Novartis Institutes for BioMedical Research and Kereos) and technical advisor to a number of funding agencies (BioAdvance Corporation, Bi-national Science Foundation, Medical Research Council and Swiss National Science Foundation). Dr. Winter is a member of the Tau Beta Pi Engineering Honor Society and several medical imaging societies, including the International Society of Magnetic Resonance in Medicine, the Society of Cardiovascular Magnetic Resonance, the Society for Molecular Imaging and the Biomedical Engineering Society. While at Washington University, Dr. Winter has collaborated with faculty from the schools of engineering, medicine and physics, as well as industrial partners including Philips Healthcare, Bristol-Myers Squibb and Dow Chemical. He has extensive publications including peer-reviewed journal papers, book chapters and scientific abstracts. Dr. Winters current active grant funding is in the area of molecular and biomedical imaging from the NIH as well as private sources (hospital foundations and American Heart Association). Dr. Winter has served as a manuscript reviewer for more than 15 scientific journals, including Cancer Research, Nature Clinical Practice, Circulation, Magnetic Resonance in Medicine, Molecular Imaging, Journal of the American College of Cardiology: Cardiovascular Imaging, Journal of the American Chemical Society and Nanomedicine.

Dr Winter was selected for this panel for his expertise in molecular imaging.
Disclosure: None.

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Appendix B

Chromium Science Advisory Board Review of *Research Framework for Evaluating the Potential Mode(s) of Action Underlying the Carcinogenicity of Hexavalent Chromium Following Exposure in Drinking Water*

July 28-29, 2009

Overview of Peer Consultation Process, List of Attendees, Agenda, and Presenter Biographical Sketches

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Overview of the Review Process

Background

Toxicology Excellence for Risk Assessment (*TERA*) was engaged as an independent third party to convene a science advisory board (SAB) panel to provide review and guidance on the investigation of the potential mode(s) of action of hexavalent chromium underlying the carcinogenic response observed in rats and mice following exposure in drinking water. The purpose of the panel review was to evaluate whether the proposed research will provide the information that is necessary to adequately determine the mode(s) of action based on the US EPA Guidelines for Carcinogen Risk Assessment (2005). ToxStrategies is coordinating the research program and the studies are being conducted at The Hamner Institute and Charles River.

The National Toxicology Program recently completed a two-year cancer bioassay for sodium dichromate dehydrate in drinking water (NTP, 2007) and reported finding intestinal tumors in mice and oral mucosal tumors in rats following lifetime exposure to hexavalent chromium. While the NTP study provides useful information for hazard identification, it is difficult to understand the relevance of these findings for humans. Therefore, a research program was initiated to investigate the mode(s) of action underlying these tumorigenic responses in rodents in order to determine the shape of the dose response curve and the human relevance of these responses prior to the development of an oral cancer slope factor for hexavalent chromium. The overall goal of these studies was to develop an understanding of the contribution of different modes of action for hexavalent chromium across a broad range of doses in order to more accurately extrapolate the results of the NTP drinking water study to humans. While a high concentration of tumors in laboratory animals indicates some level of genotoxicity, these tumors may also be due to the presence of other cellular responses, including cytotoxicity, inflammation, and high levels of oxidative stress. These secondary responses may lead to dose-dependent transitions where Cr(III) and Cr(VI) DNA reactivity finally lead to DNA damage, mutation, and transformation. The contributions of these various pathways over the range of doses and concentrations was determined by a combination of genome-wide microarray analyses in intact animals, high content imaging of activation of key DNA-damage pathways, and consideration of dose dependences in dosimetry.

The primary tasks for the SAB at this meeting were to ensure that the correct questions regarding mode of action are being asked, that all possible modes of action are being considered, that the individual studies are being designed to adequately address the questions being asked, and that the studies will provide sufficient information to satisfy the US EPA (2005) cancer guidelines regarding establishment of a mode of action and determination of the appropriate approach for low-dose extrapolation.

The SAB Review Panel

The SAB panel included seven scientists who have expertise in the key disciplines and areas of concern to review the research program. Each panelist is a well-respected scientist in his or her field. Collectively, the panel has expertise in chromium risk assessment and mode of action

research, regulatory mode of action analysis, biostatistics, physiologically based pharmacokinetic modeling, genotoxicity, oxidative stress and DNA damage, genomics, proteomics, transcriptional analysis, and magnetic resonance imaging. Panel members are very familiar with U.S. guidance and methodologies for cancer mode of action analysis. *TERA* was solely responsible for the selection of the panel members.

Each panel member disclosed information pertinent to evaluating potential conflicts of interest and biases related to the SAB as well as the organizers, sponsors, and authors of the research proposal. *TERA* carefully evaluated this information when selecting panel members. Short biographical sketches and disclosure statements for panel members are provided above.

Review Package and Charge to Peer Reviewers

The panel was sent the research proposal and review materials approximately six weeks prior to the meeting to ensure adequate time to carefully review the document and prepare for the meeting discussions. *TERA* developed a "charge to peer reviewers" document that outlines the key questions and scientific issues that need to be discussed by the panel in order to evaluate the quality and completeness of the risk assessment. Panel members provided preliminary comments several weeks prior to the meeting for the authors to consider in preparation for the meeting. As these comments were preliminary and panelists may have changed their opinion upon further review and discussion, they were not be distributed further or made part of the official meeting record.

Meeting Procedures

The meeting was organized to make the best use of the time available to hear and discuss the opinions of the panelists regarding the charge questions and the research proposal. The meeting began with brief panel introductions and a discussion of conflict of interest and bias issues. The panel discussions started with a review of the proposed mode of action hypothesis and was followed by evaluation of the individual studies that make-up the research proposal. To start each discussion section, the authors of the research proposal made a short presentation. These presentations highlighted the salient points and focused on important issues. There was a brief period for panel member clarifying questions and then the panel discussed the relevant charge questions.

Observers

The meeting was open to the public and we encouraged individuals or organizations with expertise in hexavalent chromium toxicity and risk assessment to attend the meeting as observers and submit comments on the proposed research plan. Observers submited written comments and made oral comments during the meeting. Observers were asked to submit written comments via the meeting web page by July 17, 2009. Any written comments received were distributed to the panel and to the sponsors and authors. To insure the panel's independence, observers were asked

to refrain from initiating discussions about the research proposal or related issues with the panel members.

Meeting Report

TERA drafted a meeting report that briefly summarized the panel's discussions and recommendations. The meeting report serves as a record of the peer review and will assist the authors in making revisions to the research proposal. The report will be reviewed by the panel members for accuracy before it is finalized.

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List of Attendees

Mr. Bruce Allen
Bruce Allen Consulting

Dr. Mel Anderson
The Hamner Institute for Health Sciences

Dr. Deborah Barsotti
MACTEC Engineering and

Mr. B.B. Blevins*
California Strategies, LLC.

Dr. Susan Borghoff
ILS, Inc.

Dr. Heather Burleigh-Flayer*
PPG Industries and Technology, Inc.

Dr. Jerry Campbell
The Hamner Institute for Health Sciences

Mr. Enrique Castro
Tierra Solutions, Inc.

Dr. Harvey Clewell
The Hamner Institute for Health Sciences

Dr. Darol Dodd
The Hamner Institute for Health Sciences

Ms. Shannon Ethridge*
Texas Commission on Environnemental Quality

Ms. Helen Fahy*
Fahy Associates c/o Honeywell

Dr. Lynne Haber
Toxicology Excellence for Risk Assessment (*TERA*)

Dr. Mark Harris
ToxStrategies, Inc.

Dr. Laurie Haws
ToxStrategies, Inc.

Ms. Lindsey Jones*
Texas Commission on Environnemental Quality

Dr. Vinod Kaimal*
Charles River Laboratories

Dr. Brian Mayes*
General Electric Global Research Center

Dr. Patrick McConville*
Charles River Laboratories

Dr. Glenda Moser
ILS, Inc.

Ms. Deborah Proctor
ToxStrategies, Inc.

Dr. Les Recio
ILS, Inc.

Dr. Rita Schoeny*
U.S. Environmental Protection Agency

Dr. Richard Sedman
California OEHHA

Ms. Joan Strawson*
Toxicology Excellence for Risk Assessment (*TERA*)

Dr. Rusty Thomas
The Hamner Institute for Health Sciences

Dr. Chad Thompson
ToxStrategies, Inc.

Ms. Alison Willis
Toxicology Excellence for Risk Assessment (*TERA*)

*Participating via Webinar and Conference Call

Agenda

Tuesday, July 28, 2009

8:00 **Breakfast and Registration**

8:30 **Meeting Convenes**

Welcome and Logistics: Dr. Lynne Haber, *TERA*

Introductions and Conflict of Interest and Bias Disclosures: Panel

Meeting Process, Dr. Michael Dourson, Chair

8:45 **Presentation on Mode of Action Hypothesis, Clarifying Questions from Panel**

Dr. Mark Harris, ToxStrategies

9:15 **Observer Oral Comments, Clarifying Questions from Panel (if needed)**

9:30 **Panel Discussion (with mid-morning break, approximately 10:30)**

Review Mode of Action Hypothesis

12:30 **Lunch**

1:30 **Reconvene:** Complete general mode of action discussion

Presentation on Research Framework, Clarifying Questions from Panel
Dr. Harvey Clewell, The Hamner Institute

Begin discussion of study design – 90-day study[1]

3:00 **Afternoon Break**

5:00 **Summary of Day 1 Discussions**

5:30 **Adjourn**

[1] Author presentations on the results of pilot studies on genomics, oral cavity scrapings, MRI, and PBPK modeling are available if requested by the panel.

Wednesday, July 29, 2009

8:00 **Breakfast and Registration**

8:30 **Meeting Convenes**

Wrap up any outstanding issues from Day 1

8:45 **Observer Oral Comments, Clarifying Questions from Panel (if needed)**

9:00 **Panel Discussion (with mid-morning break, approximately 10:30)**
Microarray analysis

12:00 **Lunch**

12:45 **Reconvene (with mid-afternoon break, approximately 2:30)**

Panel Discussion: MRI and PBPK modeling
High Content Image Analysis

Remaining outstanding issues

4:15 **Summary of Panel Conclusions and Recommendations**

4:45 **Evaluation of Meeting**

5:00 **Adjourn**

Biographical Sketches of Presenters

Harvey Joseph Clewell III, Ph.D., DABT

Harvey Joseph Clewell III is a professional research manager at CIIT at The Hamner Institutes for Health Sciences, located in Research Triangle Park, North Carolina. With more than 25 years of experience in environmental quality research, toxicology research, chemical risk assessment, and hazardous materials management, Dr. Clewell has gained an international reputation for his work in the application of physiologically-based pharmacokinetic (PBPK) modeling to chemical risk assessment. Pharmacokinetics is the activity of drugs or chemicals in the body over a period of time, including the processes of absorption, distribution, localization in tissues, biotransformation and excretion. Dr. Clewell has played a major role in the first uses of PBPK modeling in cancer and non-cancer risk assessments by the United States Environmental Protection Agency (EPA)**,** the Agency for Toxic Substances and Disease Registry (ATSDR)**,** the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA).

At The Hamner Institutes, Dr. Clewell oversees the development of methods for combining state-of-the art modeling of tissue dosimetry and cellular response with targeted experimental studies, in order to reduce the uncertainty in estimates of the effects of environmental chemicals and pharmaceuticals in humans. He conducts research to foster the incorporation of chemical specific and biological information in human health and safety assessments. Dr. Clewell's physiologically-based pharmacokinetic model of methylmercury — a bioaccumulative environmental toxin — has been used to estimate undesirable exposures in women of childbearing age in the U.S. Today EPA and FDA guidelines state that consumers, particularly pregnant women, women planning to become pregnant, nursing mothers and young children, should avoid fish with high levels of methylmercury and limit their intake of fish with moderate levels of the toxin. Besides methylmercury, other chemicals for which Dr. Clewell has developed models include: acetone, arsenic, coumarin, isopropanol, manganese, methylene chloride, retinoic acid, trichloroethylene, and vinyl chloride. During his career, he has assessed the hazards of exposure to children of items ranging from plastic teething rings to wood playground structures treated with chromated copper arsenate (CCA).

A Diplomate of the American Board of Toxicology, Dr. Clewell has authored numerous scientific publications, and he is an adjunct professor at the department of toxicology, University of Louisiana, Monroe, as well as an adjunct professor at the Center for Environmental Toxicology and Technology, Colorado State University in Fort Collins, Colorado. He has provided testimony on behalf of the U.S. Navy at the public hearings on the OSHA-proposed standard for occupational exposure to methylene chloride, and testimony on behalf of the Department of Defense at the Congressional hearings regarding hazards associated with the transport of nitrogen tetroxide propellant over the highways.

Prior to his position at The Hamner Institutes, Dr. Clewell was a principal at ENVIRON Health Sciences Institute in Ruston, La., where he consulted in quantitative safety/risk assessment, dose response assessment, and pharmacokinetics. For 20 years, Dr. Clewell was an officer in the U.S. Air Force Biomedical Science Corps. He developed a new program in hazardous material pollution prevention and provided guidance on hazardous materials issues to developers of aircraft such as the Advanced Tactical Fighter, the B-2 Advanced Technology Bomber, and the National Aerospace Plane. At one time, Dr. Clewell supervised a laboratory research staff of more than 100 Air Force and on-site contractor personnel, and he managed and programmed the expenditure of over $5 million in funds per year, coordinating Air Force toxicology research with other Air Force, Department of Defense, and Federal agencies. He won the Harry G. Armstrong Award for Scientific Excellence from the U.S. Air Force in 1986.

Dr. Clewell earned a B.A. cum laude in chemistry in 1969 from Bradley University in Peoria, Ill, an M.A. in physical chemistry from Washington University in St. Louis, Mo. in 1971, and a Ph.D. in Toxicology from the University of Utrecht, the Netherlands, in 2007. He maintains society memberships in Phi Eta Sigma and Phi Kappa Phi Scholastic Honoraries, the Society for Risk Analysis, the Society of Toxicology and the American Chemical Society. In 2007, the Society of Toxicology recognized Dr. Clewell with the Arnold J. Lehman Award for a major contribution to chemical risk assessment through the application of physiologically-based pharmacokinetic (PBPK) modeling.

Deborah Proctor

Deborah Proctor is a Principal Health Scientist with ToxStrategies, Inc. Ms. Proctor is based in Orange County, California and oversees west coast operations for ToxStrategies. She has over 20 years of experience in the environmental health risk assessment field, specializing in human health risk assessment, applied toxicology, mode of action evaluations for chemical carcinogens, environmental chemistry, exposure reconstruction, and quantitative dose-response analysis. Ms. Proctor is a nationally recognized expert regarding the potential health risks associated with occupational and environmental exposure to chromium. She has published extensively in this field and managed research projects that are now the bases of federal and state regulatory health criteria.

Russell S. Thomas, Ph.D.

Russell S. Thomas, Ph.D. is director of the Center for Genomic Biology and Bioinformatics and senior investigator at The Hamner Institutes for Health Sciences, located in Research Triangle Park, North Carolina. The focus of Dr. Thomas' research is studying how cellular processes are altered when an organism is exposed to chemicals or drugs. The metabolic responses of animals and human patients to disease, as well as to the desirable and adverse effects of drugs and chemicals, are very complex. Specifically, Dr. Thomas develops functional genomic screens to dissect cellular signaling pathways involved in toxicity. He applies genomic, proteomic, and

metabonomic tools for identifying biomarkers, and integrates functional genomic screens with gene expression technology to identify secondary and tertiary gene expression cascades involved in toxicity.

Metabonomics is the study of metabolic responses to drugs, environmental changes, and diseases; genomics is the study of genes and their function; and proteomics is the study of proteins. Through the application of these three tools, Dr. Thomas works to find results more quickly, economically, efficiently and accurately. His research involves identifying biomarkers to characterize disease pathology, drug efficacy or drug toxicity.

Prior to his position at The Hamner Institutes, Dr. Thomas was a scientist at Kalypsys, Inc., in La Jolla, California, where he simultaneously managed the Lead Profiling and Target Discovery groups in a drug discovery operation that included both Ph.D. scientists and research associates. In earlier research efforts, Dr. Thomas co-developed a novel microarray platform of orthologous genes in humans and mice to evaluate cross-species developmental and toxicological differences in gene expression. He performed polymorphism (SNP) surveys of toxicologically and pharmacologically important genes and co-developed a bioinformatics pipeline for analyzing polymorphism related data. He has also created bioinformatic tools for performing cross-genome comparisons in a distributed computing environment (i.e., Linux cluster), and worked on database development for gene expression and sequence visualization.
The American Chemistry Council, the National Cancer Institute, the National Institutes of Environmental Health Sciences, the U.S. Army, Honeywell, Inc., and the Formaldehyde Council have all at various times awarded Dr. Thomas with grants and contracts for research. He has presented numerous keynote addresses and invited talks at scientific symposiums, forums, and conferences. Dr. Thomas maintains professional memberships in the American Association for the Advancement of Science as well as the Society of Toxicology. The author of numerous scientific articles, Dr. Thomas has served as an editorial and scientific referee for a number of professional journals, including *Pharmacogenomics, EHP Toxicogenomics, Applied Genomics and Proteomics, Chemical Research in Toxicology, Life Sciences, Toxicological Sciences, Physiological Genomics, and Reproductive Toxicology.*

Academic and professional honors of Dr. Thomas include fellowships at Colgate-Palmolive/Society of Toxicology, the National Cancer Institute, and Ciba-Geigy/Society of Toxicology. He has won awards for outstanding student presentations from Colorado State Cell and Molecular Biology and Mountain West Society of Toxicology, as well as the Kirke L. Martin Scholarship for Outstanding Graduate Student Research. Dr. Thomas was named a William J. Johnson Chemistry Scholar and received the Tabor College Divisional Science Award for Excellence.

After earning a B.A. in 1991 in chemistry from Tabor College in Hillsboro, Kansas, Dr. Thomas went on to earn an M.S. in 1993 in radioecology and health physics from Colorado State University in Fort Collins, Colorado. He earned a Ph.D. in 1997 in toxicology from Colorado State University. Dr. Thomas did his postdoctoral research in genomics and molecular biology at the McArdle Laboratory for Cancer Research in Madison, Wisconsin.

Dr. Vinod Kaimal

Dr. Vinod Kaimal holds Bachelors and Masters degrees in electrical engineering and a doctorate in Biomedical engineering with a focus on medical imaging. Prior to joining Charles River/MIR, Dr. Kaimal worked at the Cincinnati Children's Hospital on his doctoral dissertation on magnetic resonance microscopy of tumor-targeted liposomes. At Charles River Discovery and Imaging, Dr. Kaimal is responsible for the design, planning and execution of imaging studies across all imaging modalities (MRI, CT, bioluminescence and PET) and associated data analysis and reporting. He is also responsible for the development and validation of new and existing imaging technologies.

Appendix C

Chromium Science Advisory Board Review of *Research Framework for Evaluating the Potential Mode(s) of Action Underlying the Carcinogenicity of Hexavalent Chromium Following Exposure in Drinking Water*

July 28-29, 2009

Presenter Slides

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Presentation 1 – Mode of Action for Carcinogenicity of Ingested Cr(VI): Initial Considerations

Mode of Action for Carcinogenicity of Ingested Cr(VI): Initial Considerations

By: Deborah Proctor
ToxStrategies, Inc.

Presentation Overview

- Considerations regarding the MOA for ingested Cr(VI) in rodents
- Review genotoxic pathways
- Role of inflammatory response
- Information regarding relevance to humans
- Introduce critical questions for MOA/Human Relevance Framework

Objectives of MOA research

- Seek to understand and quantify:
  - Differences in MOA at different dose levels
  - Differences between target tissue MOA in rats, mice and humans
    - Kinetics
    - Dynamics
  - Develop data needed for informed risk assessment using PBPK modeling

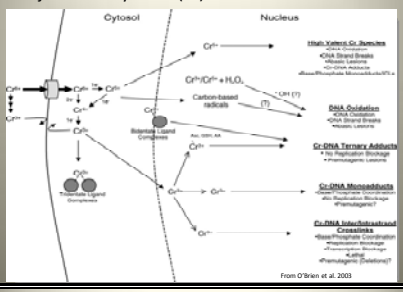
Important Points

- Cr(VI) is genotoxic in wide range of systems
  - Cr-DNA adducts, DNA single and double strand breaks, chromosomal aberrations and apoptosis
- Cr(VI) is rapidly taken into cells by non-specific ion channels
- Cr(VI) reduces to CrV, CrIV and CrIII in cytosol, endoplasmic reticulum and by mitocondria
- Reduction by glutathione (GSH), ascorbate and other reducing agents in the cell is significant
- Inter-cellular reduction generates reactive oxygen species and oxidative stress

Important Points

- Cr(VI) reduced to CrIII outside of the cell is not genotoxic because CrIII cannot readily cross the cell membrane
- Cr(VI) may act as a site of contact carcinogen producing nasal and pulmonary cancers upon inhalation and alimentary tract tumors upon ingestion
  - Puzzling that there were no non-neoplastic effects in rat oral cavity
  - Puzzling that tumors were not seen in stomach, forestomach or other GI tissues of mouse

Major Pathways in Cr(VI)-induced Genetic Lesions



From O'Brien et al. 2003

Cr Genotoxicity

Lesion	Cr Species	In Vivo	In Vitro	Number of References
DNA Adducts	Cr(V), Cr(III)	No	Yes	11
DNA single strand breaks	Cr(V)	Yes	Yes	57
DNA Protein Crosslinks	Cr(III)	Yes	Yes	33
DNA ascorbic acid links	Cr(III)	Yes	Yes	2
Oxidized bases	Cr(V)	No	Yes	11
8-oxo-dG	Cr(V)	Yes	Yes	10
Abasic sites	Cr(V), Cr(III)	No	Yes	5
DNA inter/intrastrand crosslinks	Cr(III)	Yes	Yes	17

Adapted from O'Brien et al. 2003

Inflammation

- Chronic tissue inflammation is related to increased risk of cancer in many organs including esophagus, stomach and colon
- Beaver et al. 2009 showed internasal administration of zinc chromate resulted in inflammatory response, apoptosis, and mucosal injury
- Induction of inflammation based on up regulation of Akt phosphorylation at serine 473
- Inflammation hypothesized to promote cell survival in environment of Cr(VI)-induced genotoxic injury, resulting in neoplastic progression

Inflammation

- In NTP study both rats and mice developed histiocytic infiltrates in various tissues including the liver, and chronic inflammation in the liver
- Histiocytic infiltration was reported as the predominant non-neoplastic lesions in the mouse small intestine
- Similarly in Cr(VI)-induced lung cancer, tumors in animals and humans are accompanied by overt tissue damage, irritation and inflammation

MOA Framework for Cr(VI)

Hypothesized Possible Key Events

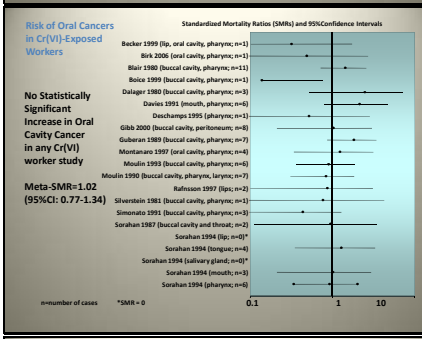
- Extracellular reduction resulting in detoxification, Competing with Absorption of Cr(VI) into cells of target tissue
- Intracellular reduction of Cr(VI)
  - Generation of oxygen radicals
  - Generation of Cr-intermediates
- Disruption of normal cellular homeostasis for Cr(III)
- DNA damage
- Apoptosis
- Inflammation
- Hyperplasia (small intestine)
- Tumors

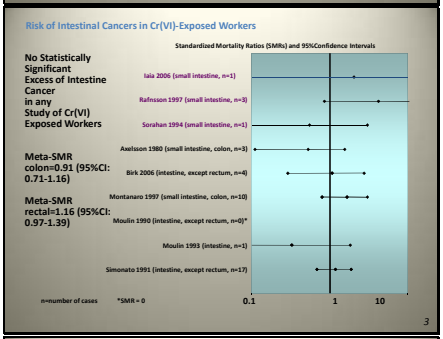
Human Relevance

- Known human carcinogen from certain occupational exposures
  - Characterized in industries with very high concentration exposures
  - Primarily in industries with exposure to partially soluble Cr(VI) such as zinc and calcium chromate
  - Lung cancers associated with significant lung burden of Cr
  - Occurs in association with highly irritating exposures
    - Affects on nasal mucosa
    - Bronchitis

Human Relevance

- Conflicting data regarding stomach cancer among Chinese villagers (Beaumont et al. 2008, Kerger et al. 2009)
  - Epidemiological data are highly limited
    - Ecologic data with very uncertain exposures
    - Stomach cancer mortality evaluated 4-13 years after initial exposure, inadequate for expected latency
    - Several potential confounding and biasing factors
- Other ecologic studies are negative, but also highly limited.

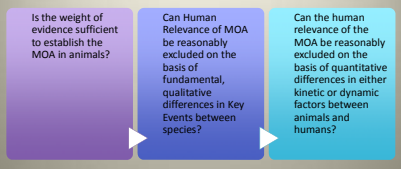




In Vivo Genotoxicity Data among Cr(VI)-Exposed Workers

- Few studies report increased MN in buccal cells
  - Benova et al. 2002 Cr(VI) Platers
  - Danadevi et al. 2004 Welders
- Several studies report DNA lesions in circulating blood lymphocytes
  - CA, SCE, DPX, MN, strand breaks
- But there are no reports of cancer in oral mucosa or lymphatic system among workers

MOA/Human Relevance



Source: ILSI/IPCS Frameworks

MOA Framework for Cr(VI)

Hypothesized Possible Key Events

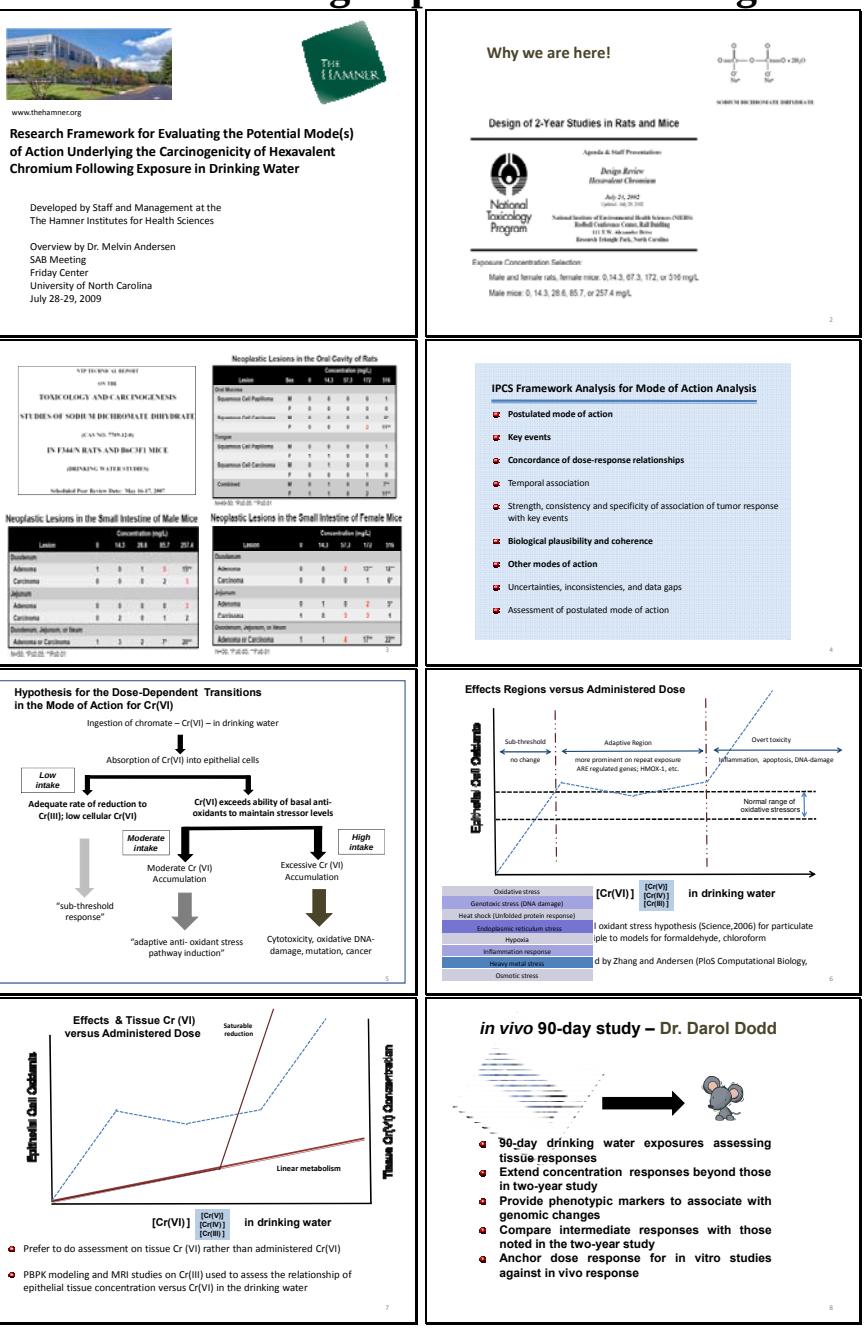
- Extracellular reduction resulting in detoxification, Competing with Absorption of Cr(VI) into cells of target tissue
- Intracellular reduction of Cr(VI)
  - Generation of oxygen radicals
  - Generation of Cr-intermediates
- Disruption of normal cellular homeostasis for Cr(III)
- DNA damage
- Apoptosis
- Inflammation
- Hyperplasia (small intestine)
- Tumors

Human Relevance…Identifying Important Risk Assessment Data Gaps

- In the event that human relevance cannot be discounted, proceed with the risk assessment:
  - Implications of kinetic and dynamic data for dose-response assessment
  - Use information quantitative differences in key events or modifying factors such that default values for uncertainty factors for species or individual differences can be chemical-specific

Based on Boobis, 2008 SOT CE Course Presentation

Presentation 2 – Research Framework for Evaluating the Potential Mode(s) of Action Underlying the Carcinogenicity of Hexavalent Chromium Following Exposure in Drinking Water



Genomics – Dr. Russell Thomas


- in vivo studies with gene expression microarray analysis on target tissues
- Fewer doses and time points (7 and 90 days)
- Capable of examining changes across the entire transcriptome
- Grouping genes based on cell function allows understanding the doses at which different cellular functions are affected
- Analyze genes for departure from linearity and threshold

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in vivo mutation – Dr. Harvey Clewell with Dr. Martha Moore, NCTR

A→ T
G→ C

- Evaluate biological marker for cancer risk
- Assess mutation changes in target tissues from animals in the 90-day study
- Establish dose-response in relation to activation of gene pathways
- Compare with oxidative stress and DNA damage markers from genomic studies

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PBPK Modeling – Dr. Harvey Clewell


- in vivo studies to assess conversion of chrome (VI) to chrome (III)
- Accounting for the essential, ubiquitous nature of chromium in test animals
- Evaluating pathways of epithelial uptake in oral cavity and intestines
- Consider enhancing the current PBPK model for chromium

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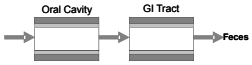
- PBPK Models similar to those of airways for inhaled irritant gases and for ingestion of vinyl acetate (another compound causing oral cavity tumors)



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Expected Outcomes based on Proposed MOA:

Dose (ppm - water)	Histopathology in life study	Gene expression patterns in life study	in vivo mutation in life study	Tissue Concentration (MRI & PBPK Modeling) in life and modeling	HCA-Cellular phenotypes in vitro studies
0.0	-	-	-	Basal tissue Cr(VI) Basal Cr(III)	naive and pretreated cells
AA	-	No significant changes	-	Predicted tissue Cr(VI) Increased tissue Cr(III)	
BB	-	No significant changes	-	Predicted tissue Cr(VI) Increased tissue Cr(III)	
CC	-	Few significant changes	-	Predicted tissue Cr(VI) Increased tissue Cr(III)	
DD	-	Stress Pathways	+/-	Predicted tissue Cr(VI) Maximum tissue Cr(III)	
EE	+/-	Apoptosis Inflammation pathways	+	Predicted tissue Cr(VI) Maximum tissue Cr(III)	
FF	++	Apoptosis, etc. & DNA-damage pathways	++	Predicted tissue Cr(VI) Maximum tissue Cr(III)	

- Integration of in life components evaluating evidence for dose-dependent transitions - phenotypic anchors, genomic GO-BMD evaluations, mutational rates and PBPK considerations
- HCA studies provide dose-response characterization for multiple endpoints and the possibility to assess likelihood of a threshold for DNA-damage (Lutz and Lutz, 2009).

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High Content Imaging Assays (HCA) Dr. Russell Thomas


- in vitro, cell-based imaging assays in multi-well plates
- Primary cells or cell lines similar to target tissue (human and rodent)
- Capable of performing large numbers of replicates, doses, and time points
- High statistical power to detect departure from linearity or threshold
- Apply to oxidative and DNA stress measures

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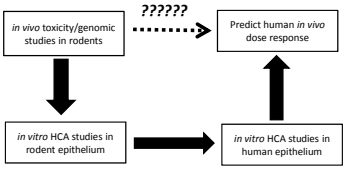
Interspecies Extrapolation



Parallelogram Approach for Using HCA for Interspecies Extrapolation of Cr(VI) dose response in Alimentary Tract Epithelial Target Cells

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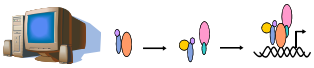
Risk Assessment – The Full Team



- Pharmacodynamics: Defining dose dependencies in gene patterns from BMD analysis
- Pharmacodynamics: Utilizing new statistical tools to establish a threshold for Oxidative stress and DNA damage/repair from HCA/Genomic studies –
- Risk Assessment: applying PBPK model and in vitro evidence for threshold to infer expected threshold for cancer response in vivo

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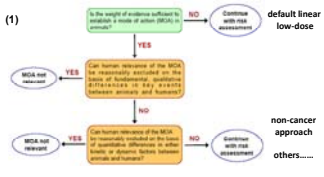
IPCS general scheme illustrating the main steps in evaluating the human relevance of an animal MOA for tumor formation

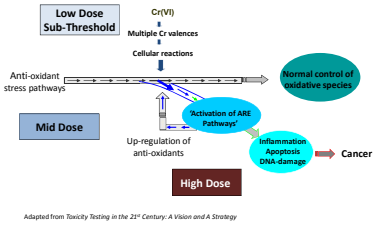


(2) Other possibilities could be considered as in the 2007 NAS report using knowledge of concentrations below those causing up-regulation of stress regulatory gene families.

17

Dose Dependent Transitions – 'Hypothesis with Cr(VI)'

Toxicity Pathway – Oxidant stress pathways and overload



Adapted from *Toxicity Testing in the 21st Century: A Vision and A Strategy*

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Additional Points of Discussion

- Time points for evaluation (genomics and histopathology)
- Including non-responding species in genomic study
- Specific in vivo mutation study, i.e., p53, TK, comet assay, or HPRT
- Value of MRI for assessing rates of reduction; are there other possibilities
- Range of endpoints to assess through HCA analysis
- Using both pretreated and naïve cells (HCA)

- Thank you for your efforts as part of this review
- You will have the group here to answer questions throughout
- Any questions, now?
- Dr. Clewell will talk about the PK work later

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Mode of Action Narrative for Cr(VI)

- Cr(VI) is a pro-oxidant with the capacity to cause oxidative stress, tissue damage, DNA-damage, gene mutation, and cancer with prolonged treatment of high concentrations in drinking water – **probably as true for you and me as for rats and mice.**
- Mammalian cells have both basal and inducible anti-oxidant stress pathways to control oxidative stress (after all, we live daily with high concentrations of a very potent, potentially mutagenic pro-oxidant - O_2)
- With increasing levels of exposure to Cr(VI) there is progress through a series of dose-dependent transitions: (1) a sub-threshold region where the Cr(VI) adds no appreciable oxidative stress compared to basal stressor levels; (2) a second region where increasing Cr(VI) leads to adaptive responses to the increased levels of oxidant stress leading to up-regulation of stress-controlling pathways; and, (3) with saturation of induced, anti-oxidant enzymes, etc., Cr(VI) builds up to levels with broad, overt effects on proteins, DNA, and other cellular constituents leading to cell-damage, apoptosis, DNA-damage, mutation and cancer with prolonged exposure.

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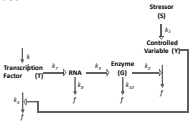
Describing feedback control in homeostatic processes and assessing transitions to loss of control

Perturbations to Cellular Responses

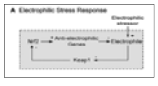
Cellular Stress Pathways

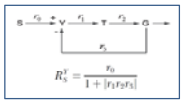
- Oxidative stress
- Genotoxic stress (DNA damage)
- Heat shock (Unfolded protein response)
- Endoplasmic reticulum stress
- Hypoxia
- Inflammation response
- Heavy metal stress
- Osmotic stress

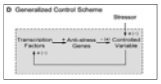


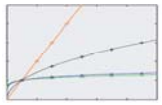
Generic schematic for negative feedback controller

Dose Response Relationship in Anti-Stress Gene Regulatory Networks









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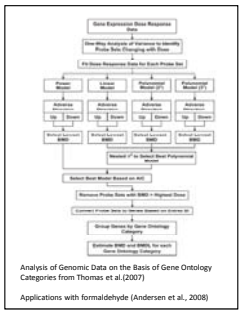

Analysis of Genomic Data on the Basis of Gene Ontology Categories from Thomas et al.(2007)

Applications with formaldehyde (Andersen et al., 2008)

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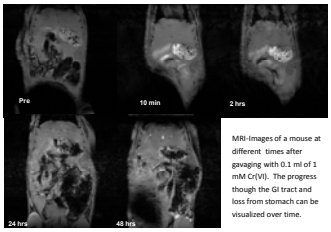
Looking at Cr(III) after Cr)VI) dosing



MRI-Images of a mouse at different times after gavaging with 0.1 ml of 1 mM Cr(VI). The progress though the GI tract and loss from stomach can be visualized over time.

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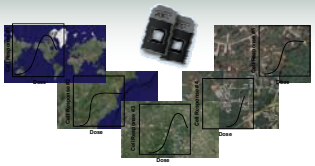
Presentation 3 – Chromium Genomics and HCA Studies

Chromium Genomics and HCA Studies

July 29, 2009
TERA Review

Russell Thomas
The Hamner Institutes for Health Sciences

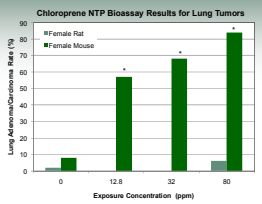
Why Genomics?



In depth of broad genomic transcriptomic analysis can provide insight at what dose global perturbations occur and the underlying biology of dose-dependent transitions

Example Application to Mode-of-Action Risk Assessment Studies

Background



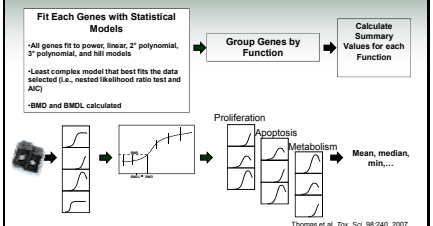
- Chloroprene is metabolized to two epoxide metabolites
- The rate of metabolism and generation of the epoxide metabolites is ~10X higher in mice than in rats.

Experimental Design for Chloroprene Cross-Species Genomics Study

Mice		Rats	
Inhalation Conc (ppm)	Internal Dose (umol/g/day)*	Inhalation Conc (ppm)	Internal Dose (umol/g/day)*
0.3	0.008	5	0.012
3	0.077	30	0.088
13	0.316	90	0.363
90	1.163	200	1.211

*Estimated total amount metabolized using PBPK model

Application of Benchmark Dose Methods to Transcriptomic Data

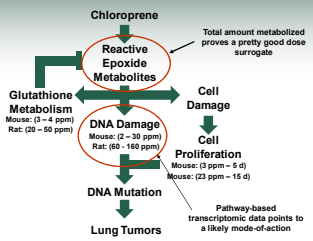


Thomas et al. *Tox. Sci.* 98:240, 2007
Yang et al. *BMC Genomics* 8:387, 2007

Cross-Species Analysis of Transcriptional BMD Values for KEGG Pathways

	Mouse			Rat		
KEGG Pathway	Genes with BMD	Median BMD (ppm)	Internal Dose (umol/g)	Genes with BMD	Median BMD (ppm)	Internal Dose (umol/g)
5 Day Time Point						
Glutathione metabolism	30	2.80	0.362	11	73.20	1.365
Mismatch repair	5	15.63	1.870	13	102.94	2.200
Nucleotide excision repair	12	40.84	4.126	26	86.39	1.715
Base excision repair	16	42.17	4.219	20	69.64	1.275
15 Day Time Point						
Glutathione metabolism	23	4.73	1.328	14	32.20	1.054
Mismatch repair	6	6.37	1.773	5	72.67	2.972
Nucleotide excision repair	15	19.46	5.002	8	72.02	2.936
Base excision repair	9	1.94	0.554	5	164.10	9.742

Proposed Mode-of-Action Model



Example Application to Non-Mode-of-Action Risk Assessment

Experimental Design for Assessing Dose-Response Changes in Chemical Carcinogens

Select Previously Tested Chemicals
4 Liver Tumorigens
2 Lung Tumorigens
(NOAEL and LOAEL)

Expose Animals for 90 Days in 5 Point Dose Response

Measure Transcriptional Changes in Target Tissue

Examine Pathway-based Dose Response Changes in Expression

Selected Chemicals for Transcriptomic Dose-Response Studies

Chemical	Route	Doses	Time Point	Target Tissue
1,4-Dichlorobenzene[#]	Gavage	100, 300, 400, 500, 600 mg/kg	90 d	Liver
Propylene glycol mono-t-butyl ether[#]	Inhalation	25, 75, 300, 800, 1200 ppm	90 d	Liver
1,2,3-Trichloropropane[#]	Gavage	2, 6, 20, 40, 60 mg/kg	90 d	Liver
Methylene Chloride[#]	Inhalation	100, 500, 2000, 3000, 4000 ppm	90 d	Liver, Lung
Naphthalene[#]	Inhalation	0.5, 3, 10, 20, 30 ppm	90 d	Lung

[#]Female B6C3F1 mice

Experimental Design for Assessing Dose-Response Changes in Chemical Carcinogens

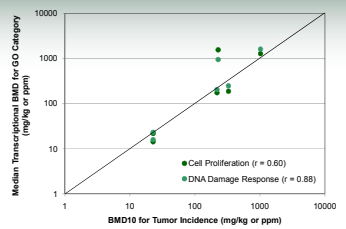
Select Previously Tested Chemicals
4 Liver Tumorigens
2 Lung Tumorigens
(NOAEL and LOAEL)

Expose Animals for 90 Days in 5 Point Dose Response

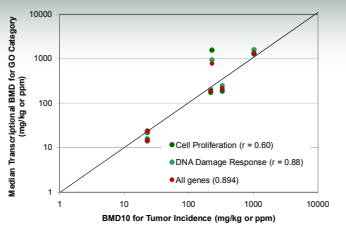
Measure Transcriptional Changes in Target Tissue

Examine Pathway-based Dose Response Changes in Expression

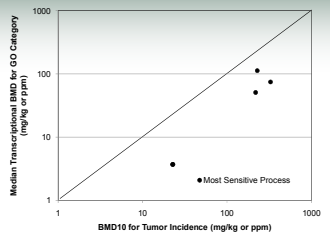
Correlation Between Tumor and Transcriptomic BMD Values



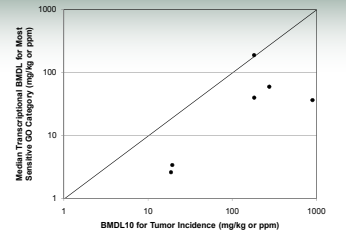
Correlation Between Tumor and Transcriptomic BMD Values



Benchmark Dose Values for the Most Sensitive Biological Processes



Benchmark Dose Values for the Most Sensitive Biological Processes



Application to Risk Assessment

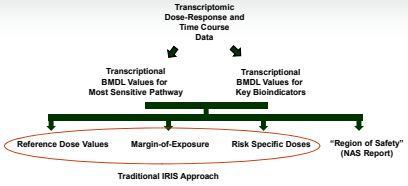
Known Mode-of-Action

Transcriptomic Dose-Response/ Time Course Data → Responses in Key Pathways → Transcriptional BMDL Values (based on target tissue dose) → Transcriptomic-based Risk Assessment

No Defined Mode-of-Action

Transcriptomic Dose-Response/ Time Course Data → All Responses → Transcriptional BMDL for Most Sensitive Pathway (based on target tissue dose) → Transcriptomic-based Risk Assessment

How Do We Implement a Transcriptomic-Based Risk Assessment?



What Does It Look Like For Non-Cancer Endpoints?

Comparison of Transcriptional BMDL Values with IRIS RfD and Margins of Exposure

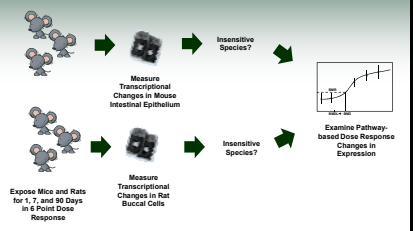
	Endpoint	Species	IRIS NOAEL or LOAEL (mg/kg/day or mg/m3)	UF	IRIS RfD (mg/kg/day or mg/m3)	Transcriptional BMDL (mg/kg/day or mg/m3)	Transcriptional RfD (mg/kg/day or mg/m3)	RfD Ratio	Human Exposure (ug/kg/day or ug/m3)	MOE
Methylene Chloride	Liver Tox	Rat	6	100	0.06	28.5	0.285	4.75	13	9,700
Naphthalene	Nasal Tox	Mouse	9.3	3000	0.003	10.5	0.0035	1.2	0.95	11,000
Trichloropropane	Clin Chem	Rat	0.7	1000	0.006	2.4	0.0024	0.42	0.008	420,000
Dichlorobenzene						39.9			0.5	79,800

What Does It Look Like For Cancer Endpoints?

Comparison of Transcriptional BMDL Values with IRIS Cancer Risk Levels

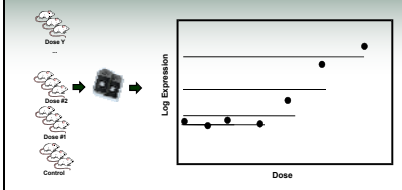
Chemical	Tumor BMDL10 (mg/kg or ppm)	Most Sensitive Pathway BMDL (mg/kg or ppm)	Transcriptional 10-6 RSD Value (mg/kg or ppm)	Transcriptional 10-6 RSD Value (Units)	IRIS 10-6 RSD Value
Naphalene (lung)	18.77	2.8	0.000028		
Methylene Chloride (lung)	182	189.5	0.001895	6.6 ug/m3	2 ug/m3
Methylene Chloride (liver)	899.9	36.4	0.000364	1.3 ug/m3	
Dichlorobenzene (liver)	182.5	39.9	0.000399		
Propylene Glycol t-Butyl Ether (liver)	275.3	59.6	0.000596		
Trichloropropane (liver)	19.4	3.39	0.0000339		

Application to Chromium



Threshold and Non-Linear Analysis

Identifying Genes With Threshold-Like Behavior

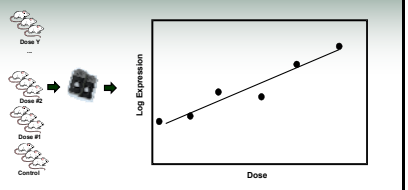


5 doses + control (n = 4 - 8 animals per dose)

$X^2 = -2 * [(LL_{flat} + LL_{rest}) - LL_{max}]$

Sequential LLR Tests
- p > 0.7 => flat
- p < 0.05 => non-flat

Identifying Genes With Non-Linear Behavior



5 doses + control (n = 4 - 8 animals per dose)

$X^2 = -2 * (LL_{Linear} - LL_{max})$

p < 0.05 => non-linear
FDR adjusted

Functional Analysis of Threshold-like and Non-Linear Genes

Test For Significance from "Zero"

Probes in GO Category with Threshold/Non-Linear Behavior and BMD	A	0
Probes in GO Category with BMD	B	D

Probes in GO Category with BMD

$$p = \binom{a+b}{a}\binom{c+d}{c} \Big/ \binom{n}{a+c}$$

Fisher's Exact Test with False Discovery Rate Correction for Multiple Comparisons

Functional Analysis of Threshold-like and Non-Linear Genes

Test For Enrichment or Reduction

Probes in GO Category with Threshold/Non-Linear Behavior and BMD	A	C
Probes in GO Category with BMD	B	D

Probes on Array with Threshold/Non-Linear Behavior and BMD

Probes on Array with BMD

$$p = \binom{a+b}{a}\binom{c+d}{c} \Big/ \binom{n}{a+c}$$

Fisher's Exact Test with False Discovery Rate Correction for Multiple Comparisons

Experimental Design

Species	Chemical	Doses	Time Point	Model System
Rat	TCDD	0.00001, 0.0001, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 10, 100 nM	24 hr	Primary Hepatocytes
Human	TCDD	0.00001, 0.0001, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 10, 100 nM	24 hr	Primary Hepatocytes

- n = 5 rats or humans per dose
- Expression analysis performed Agilent whole genome microarrays

Experimental Design

Threshold-like Probes with BMD (%)

Chemical	Species	0.7/0.05 Criteria	0.9/0.05 Criteria	Non-Linear Probes with BMD (%)
TCDD	Rat	24.9 (824/3312)	17.8 (590/3312)	14.2 (470/3312)
TCDD	Human	19.8 (212/1070)	14.0 (150/1070)	3.5 (37/1070)

Gene Ontology Results from Threshold Analysis

Rat

GO Category	Probes with BMD	Probes with Thresold	%	Fisher's Exact p-value (diff from 0)	FDR Adjusted p-value	Fisher's Exact p-value (Enrichment)	FDR Adjusted p-value
dibenzo-p-dioxin metabolic process	5	4	80.0	0.02	0.15	0.004	0.15
response to chemical stimulus	827	96	11.6	6.76E-31	6.69E-29	3.17E-08	7.75E-06
positive regulation of cell proliferation	100	19	19.0	7.42E-07	1.51E-05	0.8	1
regulation of programmed cell death	186	50	26.9	1.96E-17	1.10E-15	0.001	0.08

Human

GO Category	Probes with BMD	Probes with Thresold	%	Fisher's Exact p-value (diff from 0)	FDR Adjusted p-value	Fisher's Exact p-value (Enrichment)	FDR Adjusted p-value
dibenzo-p-dioxin metabolic process	3	3	100.0	0.05	0.5	0.008	0.7
response to chemical stimulus	61	17	27.9	2.09E-06	7.87E-05	0.13	1
positive regulation of cell proliferation	28	8	28.6	0.002	0.04	0.23	1
regulation of programmed cell death	48	8	16.7	0.003	0.05	0.7	1

Gene Ontology Results from Non-Linear Analysis

Rat

GO Category	Probes with BMD	Non-linear Probes	%	Fisher's Exact p-value (diff from 0)	FDR Adjusted p-value	Fisher's Exact p-value (Enrichment)	FDR Adjusted p-value
dibenzo-p-dioxin metabolic process	5	5	100	0.004	0.04	5.65E-05	0.005
response to xenobiotic stimulus	15	11	73.3	2.50E-05	4.32E-04	3.36E-07	8.23E-05
positive regulation of cell proliferation	100	23	23	2.86E-08	8.36E-07	0.02	0.24
regulation of programmed cell death	186	48	25.8	1.09E-16	6.92E-15	1.82E-05	0.002

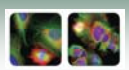
Human

GO Category	Probes with BMD	Non-linear Probes	%	Fisher's Exact p-value (diff from 0)	FDR Adjusted p-value	Fisher's Exact p-value (Enrichment)	FDR Adjusted p-value
dibenzo-p-dioxin metabolic process	3	3	100	0.05	1	3.82E-05	0.01
response to xenobiotic stimulus	6	3	50	0.09	1	7.10E-04	0.21
positive regulation of cell proliferation	28	0	0	1	1	0.6	1
regulation of programmed cell death	48	1	2.1	0.5	1	1	1

High Content Imaging Studies

Design of High Content Studies





- **Cell lines:**
  - **M-ICc12 mouse intestinal epithelial cells**
  - **Caco-2 human colon epithelial cells**
  - **Primary cells from target sites possible, but would require considerable method development**
- **Expose cells to 20 point DR and 3 time points (6, 24, and 72 hrs) AND multiple replicates**
- **Measure DNA damage and oxidative stress endpoints**
  - **H2AX foci (DSB)**
  - **53BP1 foci (DSB, SSB, alkylating agents, UV)**
  - **Nuclear phos-p53/total p53 (DSB, base damage)**
  - **Micronuclei (chromosomal damage)**
  - **Conversion of dihydroethidium to ethidium (Ox stress)**
  - **Nuclear phos-ATM**
  - **Nuclear phos-Chk2**

Presentation 4 – Chromium VI and III PBPK Model

Chromium VI and III PBPK Model

Harvey Clewell and Jerry Campbell

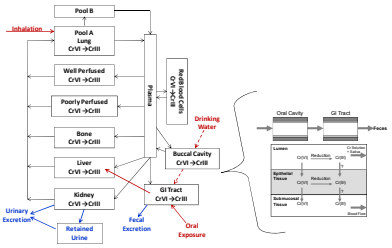
July 28-29, 2009

Model Development

- Update O'Flaherty rat model to run in acslX
- Scale by bodyweight for mouse extrapolation
- Incorporate description of the oral cavity and intestinal lumen based on vinyl acetate model
- Use model to predict blood and kidney concentrations from NTP drinking water studies

Model Structure

(based on O'Flaherty model)



Physiological Parameters

Parameter	Mouse	Rat	
QCC	16.5	15.0	Cardiac output (L/h/kg)
Fraction of Cardiac Output			
QLC	0.02	0.25	Liver
QKC	0.17	0.17	Kidney
QIC	0.141	0.17	GI Tract
QBC	0.03	0.03	Bone
QOCC	0.01	0.01	Oral Cavity
Oral Cavity			
SAOC	2.5	5	Surface Areas (cm2)
VLOC	1.2	2.4	Volumes (mL)
WLO	0.006	0.006	Lumen Thickness (cm)
WTO	0.01	0.01	Tissue Thickness (cm)
WXO	0.02	0.02	Sumucosal Thickness (cm)
FRACS	0.43	0.43	Saliva flow (fraction of ingestion rate)
Tissue volume (fraction of BW)			
VLC	0.055	0.04	Liver
VKC	0.0167	0.01	Kidney
VIC	0.0422	0.03	GI Tract
VRBCC	0.03	0.03	RBC
VWC	0.2	0.2	Well perfused (less liver, kidney and GI)
VPC	0.7	0.7	Slowly perfused (less bone)

Rate of CrVI and CrIII absorption from GI tract

* non-linear absorption
* needed to fit NTP 2007 tissue data

$$KGIi = KGIiI * \frac{KMOGIi}{KMOGIi + CGIi}$$

where: KGIi (L/h) is the clearance for Cr from the GI tract
KGIiI (L/h) is the maximal clearance for GI uptake of Cr (L/h)
KMOGIi is the transporter affinity and CGIi is the Cr concentration in the GI tract.

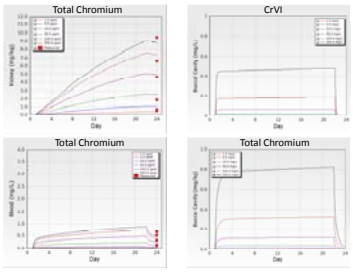
Oral absorption rates for CrVI and CrIII
(fit to NTP, 2007 kidney data).

Parameter	Mouse	Rat
Maximum Clearance (mg/kg/h)		
KGI6IC	0.00045	0.00025
KGI3IC	1.03E-05	1.03E-05
Affinity for transporter (mg/L)		
KMOGI6	5.6	9.5
KMOGI3	12.5	10.5
Buccal Cavity Absorption Rates (L/h/kg)		
Lumen to GI		
KGIOC6C	0.703	0.703
KGIOC3C	0.703	0.703
Lumen to Tissue		
KLTOC6C	4.07E-02	4.07E-02
KLTOC3C	4.07E-03	4.07E-03
Tissue to Submucossal Tissue		
KTXOC6C	1.16E-05	1.16E-05
KTXOC3C	2.16E-06	2.16E-06

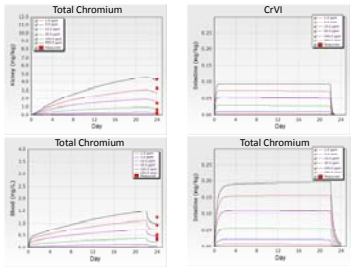
Summary: Initial Model Development

- Expanded chromium PBPK model to include descriptions of bioassay target tissues
  - buccal cavity and GI tract
- Allometric scaling of rate constants and physiological parameters
  - description of the mouse
- Incorporated non-linear uptake in intestinal compartments
  - described CrVI and CrIII dose related uptake (mouse, rat)

Simulation of the rat kidney, blood and buccal cell concentrations (NTP, 2007)
(measured 2 days after 22 days on drinking water containing sodium dichromate)

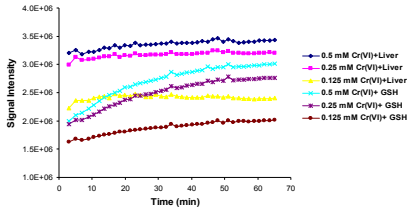


Simulation of the mouse kidney, blood and upper intestine concentrations (NTP, 2007)
(measured 2 days after 22 days on drinking water containing sodium dichromate)



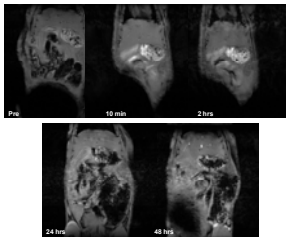
Initial MRI Results (UNC)

MR signal-time curves for Cr(VI) reduction with 190 µl liver homogenate or 5 mM GSH



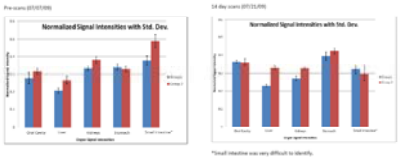
Initial MRI Results (UNC)

MR Images of a mouse at different time points after gavaging Cr(VI) (0.1 ml of 1 mM Cr(VI)).



Recent MRI Results (CRL)

MR images of mice after 14 day exposure to
516 g/L Sodium Dichromate Dihydrate in drinking water



Summary: Initial MRI Results

- Successfully detected Cr(III)
  - in vitro: from reduction of Cr(VI)
  - in vivo: following gavage of Cr(VI)
- Unable to detect increased Cr(III) in mice after 14 days of exposure to Cr(VI) in drinking water at highest dose in NTP bioassay
  - inconsistent with previous tissue Cr data at 21 days

Presentation 5 – Chromium SAB meeting

Chromium SAB meeting
July 28/29 2009

charles river
accelerating drug development. exactly.

Patrick McConville, Ph.D.
Vinod Kaimal, Ph.D.
Discovery and Imaging Services, Ann Arbor

Premise

- Cr(III) is paramagnetic and causes T1-shortening in MR images [1] but Cr(VI) is non-paramagnetic, therefore does not affect contrast in MR images.
- Cr(VI) is thought to be converted to Cr(III) in the stomach and small intestine [2]

1. GOLMAN, K.et al, "A magnetic resonance imaging contrast medium for the liver and bile", Investigative Radiology, Supplement 243/245, 1988.
2. UENO, S. et al, "Formation of paramagnetic chromium in liver of mice treated with dichromate(VI)", Toxicology and Applied Pharmacology, 135, 165/171, 1995.

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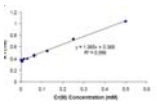
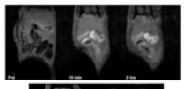
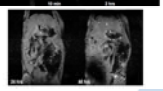
Results from studies conducted at UNC

Relaxivity measurements in solution



Imaging after oral dosing (0.1ml of 1mM Cr(VI)





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Results from studies conducted at UNC

- In vitro relaxivity measurements of Cr(III) suggest that, at 9.4T
  - Relaxivity of Cr(III) ~ 1.37mM^{-1}s^{-1} at 9.4T
  - Compare with about 3.79mM^{-1}s^{-1} for Gd based contrast agents.
  - 0.1mM Cr(III) causes a 30% increase in relaxivity
    - approximate limit of detection at 9.4T in tissue.
- In vivo
  - Early in vivo pilot studies (UNC) with oral gavage of 1mM Cr(VI) indicate some increase in signal in abdomen but several questions remain
    - Ability to image chronic Cr accumulation and oxidation not addressed
    - Animal to animal variability not addressed
    - Contrast enhancement of tissues other than stomach not addressed

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Pilot study-design (CRL DIS Ann Arbor)

- 3 groups of 5 animals each.
  - Group 1:Controls (pure drinking water)
  - Group 2: High dose (516mg SDD(Cr(VI)/L in drinking water)
  - Group 3: Low dose (172mg SDD(Cr(VI)/L in drinking water)
- Full body T1 weighted scans:
  - TR=500ms
  - TE=12.5ms
  - FOV= 70mm x 30mm x 12 slices
  - resolution = 270µm x 250µm x 1mm
- 3 reference vials within field of view containing a tissue equivalent agarose phantom
  - 2% agarose and 1mM, 0.5mM and 0.25mM Cr(III)
- Pre-scans and Day 14 scans (completed last week)

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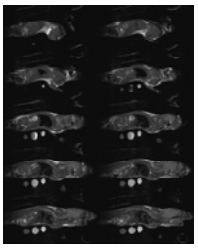
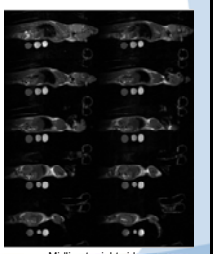
Example images



Left side to midline (sagittal)



Midline to right side (sagittal)

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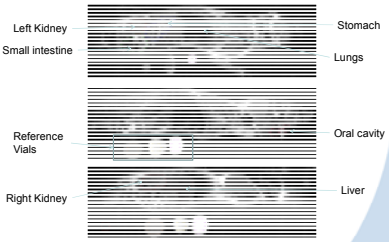
Image segmentation



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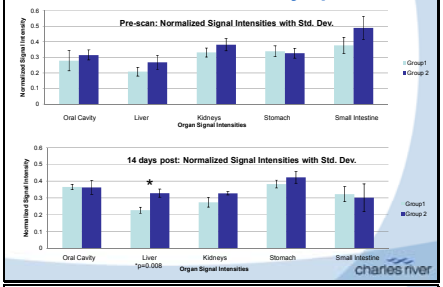
Results: Pre-scan vs 14 days post



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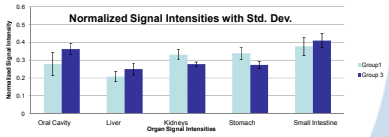
Results: Group 3 - low dose (14 days post)

Group 1(pre-scans) & Group 3 Day 14(Low dose: 2 animals only)



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Summary

- Tube references suggest 0.1-0.2mM Cr(III) is approximate LOD at 7T
- No substantial contrast enhancement has been observed (Day 14)
  - Published data suggests [Cr] tissue levels should be beyond the LOD
    - What proportion of total [Cr] is the paramagnetic form [Cr(III)] ?
  - Need to firstly quantify [Cr] in each tissue analytically to interpret the data and determine path forward
  - May need to refine or optimize the dosing or imaging protocol based on tissue analyses
    - Small intestine is challenging to identify and segment
    - Use of surface coil for increased sensitivity and enable higher resolution in intestine ?
      - Need intestine [Cr] first
- May need larger sample size to quantify differences between groups over inherent variability from animal to animal.
- 0.1mM Cr = 5.2µg Cr/g of issue
  - must be Cr(III) for signal modulation
  - Ideally need to resolve Cr(III) from Cr(VI) in extracted tissue

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Appendix D

Chromium Science Advisory Board Review of *Research Framework for Evaluating the Potential Mode(s) of Action Underlying the Carcinogenicity of Hexavalent Chromium Following Exposure in Drinking Water*

July 28-29, 2009

Additional Presentations

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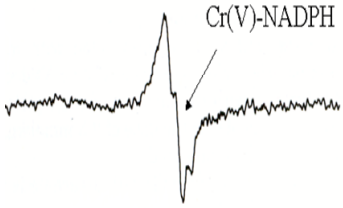
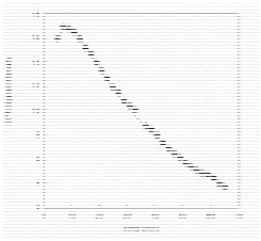
Presentation Slides – Presented by Panel Member Dr. Shi





Cr(V) generation on the skin of rat