Report of the Nickel Ion Bioavailability Workshop

Volume II

February 15-16, 2010
Northern Kentucky University METS Center
Erlanger, Kentucky

Peer Consultation Organized by:
Toxicology Excellence for Risk Assessment
(http://www.tera.org/peer/)

Contact: haber@tera.org

May 27, 2010

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Appendix A: Meeting Materials

Workshop on Nickel Ion Bioavailability

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Panel Biographical Sketches and Conflict of Interest

Biographical Sketches of Panel Members

Dr. Ambika Bathija has a Ph.D. in Biochemistry and post-doctoral training in toxicology at the Case Western Reserve University, University of Illinois Medical School and Harvard Medical School. She is currently a toxicologist at the U.S. EPA in the Office of Water, Office of Science and Technology, Health Effects and Criteria Division. Dr. Bathija serves as coordinator for the Federal-State Toxicology and Risk Analysis Committee (FSTRAC) and Disinfectants and Disinfection By-Products team. She has worked as a toxicologist for EPA for almost 25 years in the Office Solid Waste and Emergency Response, Office of Air and Radiation and now in the Office of Water. Dr. Bathija is a chemical manager for radionuclides, nitrate/nitrite, metolachlor, acetochlor and several others. She has worked for several years on developing the Draft Toxicity Profile for Soluble Nickel Salts for the EPA Office of Water, which is waiting to be finalized by EPA.

Dr. John Bukowski received his M.P.H. from the University of Michigan, a Ph.D. in epidemiology from the University of Medicine and Dentistry of New Jersey, and a doctorate in veterinary medicine from Michigan State University. He is a senior associate at WordsWorld Consulting, a biomedical and medical-writing consultancy. He provides research assistance on epidemiology, public/occupational health, and risk assessment, as well as general assistance on issues relating to clinical medicine. His epidemiology and public health career has spanned 20 years, including a broad base of experience within government, academia, and private industry. Dr. Bukowski's clinical research experience includes a post as Director of the Clinical Research Centre at the University of Prince Edward Island, Canada. He has most recently worked as a senior scientist and epidemiologist for ExxonMobil Biomedical Sciences, focusing on such varied topics as air pollution, health effects of solvents, children's health, reproductive health, neurological health, risk assessment, toxicity of metals, and emerging health issues. He has authored numerous peer-reviewed articles as well as a multitude of reports, critiques, reviews, and white papers. He recently served as a peer reviewer for the Texas Commission on Environmental Quality's nickel screening level in a peer review organized by TERA. Dr. Bukowski is also an Adjunct Associate Professor in the School of Medicine at Wright State University.

Dr. Harvey Clewell received his Ph.D. in toxicology from the University of Utrecht, The Netherlands, 2007. He is the Director of the Center for Human Health Assessment at the Hamner Institutes for Health Sciences. He has over twenty-five years of experience in research and consulting in the areas of environmental quality, toxicology, risk assessment, hazardous materials management application of physiologically based pharmacokinetic (PBPK) modeling to chemical risk assessment and pharmaceutical safety assessment. His current research interests include the application of PBPK modeling to the interpretation of human biomonitoring data, the incorporation of genomic dose-response information in quantitative risk assessment, and the development of biologically based dose response modeling approaches. In his position at the Hamner, and in his previous position as a principal at ENVIRON International, he has performed research for diverse sponsors, including private companies, government agencies, and trade

groups. Dr. Clewell is a Diplomate of the American Board of Toxicology. His research on nickel focused on modeling of nickel compound dosimetry and its impact on risk assessments for nickel; work conducted for EPRI and Florida Power and Light (1998-2001). He also provided comments on the CalEPA proposed Public Health Goal for Soluble Nickel for the Metal Finishing Association of Southern California, Inc. (1999-2001).

Dr. Max Costa received his Ph.D. in Pharmacology from the University of Arizona School of Medicine. He is currently Professor and Chairman of the Department of Environmental Medicine, New York University School of Medicine where he also serves as Professor of Pharmacology and Director of the Nelson Institute of Environmental Medicine. He is also the Program Leader of the Environmental and Molecular Carcinogenesis Program, NYU Cancer Institute. Dr. Costa has served on numerous expert panels and committees addressing nickel and other metals, including those for the IUPAC, IARC, ATSDR, ICOH, US EPA Science Advisory Board Committee National Academy of Sciences Committee, and NIEHS. He has served as President of the Metals Specialty Section of the Society of Toxicology He serves on editorial boards of many journals and has published several hundred journal articles, book chapters and books. Dr. Costa has conducted research and analysis on nickel with funding from NIH. He received small grant from NiPERA to study Ni ion distribution in human cells following exposure to Nickel Sulphide Particles (~ 5 years ago).

Dr. Michael Dourson has a Ph.D. in Toxicology from the University of Cincinnati. He is currently President of Toxicology Excellence for Risk Assessment (*TERA)*, an independent non-profit group. Prior to founding *TERA*, he spent 15 years with the U.S. EPA, holding several leadership roles, including chairing the Reference Dose Work Group of EPA's Integrated Risk Information System (IRIS). Dr. Dourson has co-authored dozens of chemical assessments and developed or peer-reviewed hundreds of risk values, including assessments of nickel and other metals. Dr. Dourson is a board certified toxicologist (DABT) and a Fellow of the Academy of Toxicological Sciences. He received the Society of Toxicology's Lehman award and the International Society of Regulatory Toxicology and Pharmacology's International Achievement award. He has been elected to multiple positions including President of the American Board of Toxicology, President of the Risk Assessment Specialty Section of the Society of Toxicology (SOT), and Secretary of the Society for Risk Analysis. He is a media resource specialist in risk assessment for the SOT, a member of the editorial board of two journals, and vice chair of the NSF International Health Advisory Board. Dr. Dourson was a co-author on the *TERA* nickel assessment prepared for the U.S. EPA, Health Canada and the Metal Finishing Association of Southern California, Inc. and two subsequent related publications. He also reviewed the non-cancer U.S. EPA reference doses developed in the 1980s and 1990s.

Dr. Andrea Hartwig received her Diploma in Chemistry and her Habilitation in Biochemistry in Bremen and then became Professor for Food Chemistry in Karlsruhe, Germany. She is currently Professor at the Technische Universität Berlin (Berlin Institute of Technology) and Chair of Food Chemistry and Toxicology at the Institute. Her main research area focuses on the impact of carcinogenic metal compounds and essential trace elements on genomic stability, with special emphasis on DNA damage induction and effects on DNA repair, gene expression and cell cycle control. At present Dr. Hartwig is focusing on interactions of toxic metal compounds and essential trace elements with so called zinc finger proteins involved in DNA repair, gene expression and tumor suppressor functions, including their redox regulation. Further research focuses on the toxicology of nanomaterials. She is President of the German MAK Commission,

a member of several boards of international journals, and author of about 120 scientific publications.

Dr. Uwe Heinrich received his Diploma (M.S.) in Zoology (Endocrinology, Physiological Etiology, and Histology) and doctorate in Biology (Zoology, Physiology, Biochemistry, and Pharmacology) at the Eberhard Karls University in Tuebingen, Germany. In 1984, Dr. Heinrich became a lecturer in Toxicology and Experimental Oncology at the Hannover Medical School and received his Dr. rer. biol. hum. habil. (habilitation) and venia legend in Experimental Oncology at the same university in 1989. He is currently Professor of Toxicology and Aerosol Research at the Hannover Medical School, Germany, and Director of the Fraunhofer Institute for Toxicology and Experimental Medicine. Dr. Heinrich was a research fellow at the Institute of Toxicology of the Bayer AG and at the Medical Institute of Environmental Hygiene at the Heinrich Heine University in Duesseldorf. His main research interest is focused on inhalation toxicology, environmental and occupational hygiene, risk assessment, and preclinical research. At present, he is a Fellow of the Academy of Toxicological Sciences, editor of the International Journal of Hygiene and Environmental Health, member of the Health Effects Institute Research Committee, and serves on various national and international committees. In more than 100 articles he published his research results and received the Kenneth Morgareidge Award for outstanding research in the area of inhalation toxicology.

Dr. Joseph R. Landolph, Jr., received his Ph.D. in Biophysical Chemistry from University of California at Berkeley under Professor Melvin Calvin (Nobel Laureate, member of U. S. National Academy of Sciences). His Ph.D. work involved metabolism of BaP, and its induction of cytotoxicity in cultured mouse liver epithelial cells and cytotoxicity/morphological transformation in Balb/c 3T3 mouse fibroblasts. He performed postdoctoral work under the late Professor Charles Heidelberger (member of the National Academy of Sciences) at the University of Southern California Comprehensive Cancer Center in chemical carcinogenesis. At the University of Southern California Dr. Landolph is currently Associate Professor of Molecular Microbiology/ Immunology and Pathology in Keck School of Medicine, Associate Professor of Molecular Pharmacology/Pharmacological Sciences in the School of Pharmacy, and a Member of the USC/Norris Comprehensive Cancer Center, with tenure. His research interests/activities include studies of the genetic toxicology/carcinogenicity of carcinogenic nickel (Ni), chromium (Cr), and arsenic compounds and polycyclic aromatic hydrocarbons. His laboratory studies the ability of carcinogenic Ni and Cr compounds to induce morphological/neoplastic transformation of C3H/10T1/2 mouse embryo cells, and expression of oncogenes/inactivation of expression of tumor suppressor genes and de-regulation of global gene expression, in Ni-transformed cell lines. He is an expert in chemically induced mutation and morphological/neoplastic transformation in murine/human fibroblasts. He has served on numerous scientific advisory committees for U.S. EPA, NIEHS and NCI of the U. S. NIH, the National Academy of Sciences the Air Resources Board of California (Scientific Review Panel for Toxic Air Contaminants), and the Office of Environmental Health Hazard Assessment of California's E. P. A, (Carcinogen Identification Committee). Dr. Landolph has worked in the area of nickel carcinogenesis/cell transformation/genotoxicity for many years and received support from USC, National Institutes of Health, NiPERA, and other agencies. The NiPERA research contracts have dealt with studies of the ability of nickel compounds to be phagocytosed into mammalian cells, and to induce cytotoxicity, chromosome aberrations, and morphological and neoplastic transformation of C3H/10T1/2 Cl 8 mouse embryo cells, as well as examining the relative genotoxic potentials of four samples of nickel.

Dr. Len Levy holds a doctorate in experimental pathology from the Institute of Cancer Research, London. He is Emeritus Professor of Environmental Health at Cranfield University within the Institute of Environment and Health where he previously served as Full Chair. Earlier he was Head of Toxicology and Risk Assessment at the UK Medical Research Council's Institute for Environment and Health based at the University of Leicester. Dr. Levy has conducted occupational and environmental risk assessments on many different types of substance, ranging from pesticides to metals and solvents, including recently focussing on the susceptibility of young children for lead. He has undertaken experimental research on a number of metals including nickel, chromium and cadmium. He has held academic positions at the University of Aston, where he developed courses in occupational toxicology and established an Industrial Toxicology Unit to research mechanisms and causes of occupational cancer and give advice to industry, trade unions and Government departments; and the University of Birmingham's Institute of Occupational Health where he was a Reader in Occupational Health and continued his research into causes and mechanisms of occupational cancer. He is currently an independent member on Health and Safety Commission's Working Group on the Assessment of Toxic Chemicals (WATCH) and the Advisory Committee on Toxic Substances (ACTS), and the UK nominee and vice-chair on the EU Scientific Committee on Occupational Exposure Limits (SCOEL), and is also a member of the Veterinary Products Committee (VPC). He has been an invited Working Group member to numerous International Agency for Research on Cancer (IARC) Monograph meetings and has chaired two of these meetings in recent times. Dr. Levy is a Fellow of the Faculty of Occupational Medicine and the British Toxicological Society and in 2000 was awarded the Order of the British Empire (OBE) for Services to Health and Safety. Dr. Levy conducted experimental studies in rodents with nickel in the 1980s with funding from the UK Health and Safety Executive. He was a member of the IARC Monograph meeting in 1990 – Vol. 49, which evaluated nickel.

Dr. Günter Oberdörster earned his D.V.M. and Ph.D. (Pharmacology) from the University of Giessen in Germany and is Professor in the Department of Environmental Medicine at the University of Rochester. He is Director of the University of Rochester Ultrafine Particle Center, Principal Investigator of a Multidisciplinary Research Initiative in Nanotoxicology and Head of the Pulmonary Core of the NIEHS Center Grant. His research includes the effects and underlying mechanisms of lung injury induced by inhaled non-fibrous and fibrous particles, including extrapolation modeling and risk assessment. His studies with ultrafine particles influenced the field of inhalation toxicology, raising awareness of the unique biokinetics and toxicological potential of nano-sized particles. Dr. Oberdörster has served on numerous advisory committees for government and private organizations in the U.S. Europe and Canada. He serves on the editorial boards of several journals and has won numerous awards for his work inhalation toxicology. Dr. Oberdorster has conducted research on nickel, including a grant from NiPERA in the early 1990s to study the role of indirect mechanisms of nickel toxicity in lung tumor formation: A species comparison of cell proliferation. He has published a number of papers discussing nickel toxicity and dosimetry, including several publications with Dr. Oller.

Mr. Steven K. Seilkop received his M.S. in Statistics from Miami University and has completed advanced graduate studies in statistics and biostatistics at the University of North Carolina and North Carolina State University. He currently is a consulting biostatistician for SKS Consulting Services and is an Adjunct Scientist with the Lovelace Respiratory Research Institute. He has been a statistical consultant in governmental, industrial, and academic settings for more than 30 years. Mr. Seilkop has served as Principal Investigator on statistical support contracts with the National Institute of Environmental Health Sciences (National Toxicology Program), U.S.

Environmental Protection Agency, and National Oceanic and Atmospheric Administration. He is experienced in the analysis of toxicological, epidemiological, biomedical, pharmaceutical, and environmental data and his areas of specialization include carcinogenicity testing, toxicity testing, and risk assessment. Mr. Seilkop has extensive experience related to nickel. He led a contract research team supporting the work of the International Committee on Nickel Carcinogenesis in Man (ICNCM) and was personally responsible for development and implementation of statistical analysis methods used in the report. He drafted the manuscript describing the study and its findings, which was published as the Report of the ICNCM (*Scand Work Environ Health* 16:1-82, 1990). He has several other relevant publications, including Seilkop and Oller (2003), *Respiratory cancer risks associated with low-level nickel exposure: an integrated assessment based on animal, epidemiological, and mechanistic data*; and Sivulka and Seilkop (2009), *Reconstruction of historical exposures in the US nickel alloy industry and the implications for carcinogenic hazard and risk assessments*. Mr. Seilkop is married to Ms. Donna Sivulka, who served as Executive Director of NiPERA from 1989-1995. They both have served as consultants to NiPERA and member companies of the Nickel Institute. Mr. Seilkop received research and analysis support from NiPERA on "Incorporation of Biological Information in the Risk Assessment Process," (1998) and for research investigating integration of animal, epidemiological, and mechanistic data in assessing respiratory cancer risks associated with low-level nickel exposure (2002-2003). He also has conducted research and analyses supporting reconstruction of historical exposures in the US nickel alloy industry and the Clydach nickel refinery, funded by a private company (2008-present).

Dr. Zong-Can Zhou received his MD from Beijing Medical University in 1966. He is a Professor in the Department of Toxicology, School of Public Health, Peking University Health Science Center. His research fields are cellular and molecular toxicology, environmental mutagenesis and carcinogenesis, safety evaluation and risk assessment of drug and chemicals. He is a Member of Expert Committee for human drug evaluation, State Food and Drug Administration; Member of Expert Committee for Health-related product evaluation, Ministry of Health; Member of Expert Committee for The National Medical Examination Center, Ministry of Health; Chairman of Toxicology Society of Chinese Preventive Medicine Association; Vice-Chairman of Genetic Toxicology Committee of Chinese Toxicology Society; and a member of Committee Hygiene Standard Committee of the Ministry of Health, PR China. Dr. Zhou prepared a hazard and risk assessment report for nickel compounds in co operation with the Chinese Academy of Inspection and Quarantine in 2008.

Biographical Sketches of Presenters

Dr. Julie E. Goodman, D.A.B.T. received her Sc.M. in Epidemiology and Ph.D. in Environmental Health Sciences/Toxicology from Johns Hopkins University's Bloomberg School of Public Health. She is an epidemiologist and board certified toxicologist at Gradient, an environmental consulting firm. She is also an adjunct faculty member in the Department of Epidemiology at the Harvard School of Public Health, where she co-instructs a course on systematic reviews and meta-analysis. Dr. Goodman's focus is on human health risks from chemicals in the environment and consumer products. Her primary responsibilities include the design, oversight, analysis, and interpretation of epidemiology studies, and the evaluation of chemical toxicology data, apparent disease clusters, and chemical exposures. Before joining Gradient, Dr. Goodman was a Cancer Prevention Fellow at the National Cancer Institute, where she conducted molecular epidemiology studies on colon cancer risk. She was also instrumental in the development of Polymorphism Interaction Analysis, a powerful statistical tool for cancer risk assessment. Dr. Goodman has conducted a critical review and weight-of-evidence assessment of soluble nickel compounds and respiratory cancer risk based on animal carcinogenicity studies, mode-of-action studies, and occupational epidemiological studies. Based on this work, Dr. Goodman was an invited observer at the IARC Monograph 100 Meeting C: Metals, Particles and Fibres (March 2009). Dr. Goodman is a Diplomate of the American Board of Toxicology.

Goodman, JE; Prueitt, RL; Dodge, DG; Thakali, S. 2009. "Carcinogenicity assessment of water soluble nickel compounds." *Crit. Rev. in Toxicol.* 39(5):365-417

Dr. Adriana R. Oller, D.A.B.T. Dr. Adriana Oller is originally from Argentina where she obtained a Master's degree in Biochemistry from Buenos Aires University. After completing a two-year residency in Toxicology and Forensic Chemistry at the School of Pharmacy and Biochemistry (Buenos Aires University), she emigrated for the United States where she completed a Ph.D. in Genetic Toxicology at the Massachusetts Institute of Technology (Cambridge, MA). Dr. Oller continued genetic toxicology research on spontaneous mutations and DNA repair at the Lineberger Cancer Research Center in Chapel Hill and NIEHS in Research Triangle Park. In 1994, she joined the staff of the Nickel Producer Environmental Research Association (NiPERA, Inc). During the last 15 years Dr. Oller has managed the human health research program for NiPERA, with particular emphasis on the mutagenicity and carcinogenicity effects of nickel and has been responsible for representing NiPERA at scientific and regulatory meetings worldwide. She has participated in several experts panels including the 12th Task Force on Harmonisation of Classification and Labelling: Expert Group on Carcinogenicity. She served as a member of Journal of Environmental Monitoring editorial board from 2004 until 2006. She is a diplomate of the American Board of Toxicology. A list of Dr. Oller's nickel-related publications is shown below.

Oller, A.R. Cappellini, D., Henderson, R., Bates, H.K. 2009. Temperature effect on nickel release in ammonium citrate, Journal of Environmental Monitoring 11(9), 1697-9.

Oller, A.R., Cappellini, D., Henderson, R. Bates, H.K. 2009. Comparison of nickel release in solutions used for the identification of water-soluble nickel exposures and in synthetic lung fluids, Journal of Environmental Monitoring 11(4), 823 - 829.

Oller, A.R., Kirkpatrick, D.T., Radovsky, A., Bates, H.K. 2008. Inhalation carcinogenicity study with nickel metal powder in Wistar rats. Journal of Toxicology and Applied Pharmacology. 233: 262-275.

Heim K., Bates, H., Rush, R., Oller, A. 2007 Oral Carcinogenicity Study with Nickel Sulfate Hexahydrate in Fischer 344 rats. Journal of Toxicology and Applied Pharmacology, 224: 126–137.

Oller, A., Erexson, G. 2007. Lack of micronuclei formation in bone marrow of rats after repeated oral exposure to nickel sulfate hexahydrate. Mutation Research, 626:102-110.

Ke Q., Davidson T., Kluz T., Oller, A., Costa, M. 2007. Fluorescent tracking of nickel ions in human cultured cells. Toxicol Appl Pharmacol. Feb 15;219(1):18-23.

Vincent, H.H., Feldman, J., Harrison, R., Monks, P.S., Oller A., Salbu, B., Shotyk, W. 2004. Mission, aims and scope: the audience for JEM now and in the future. Journal of Environmental Monitoring 6(6): 67N Editorial Material.

Oller, A.R. and Bates, H.K. 2003-2005. Introduction to JEM's Metals in Perspective column articles. JEM 2003 5: 31N-32N, 56N, 71N, 95N, 122N; 2004 6:14N, 36N, 74N, 104N, 145N; 2005 7:411N-412N.

Seilkop, S.K., Oller, A.R. 2003. Respiratory cancer risks associated with low-level nickel exposure: An integrated assessment based on animal, epidemiological, and mechanistic data. Regul Toxicol and Pharmacol. 37: 173-190.

Seilkop S.K., Oller A.R. 2005. Corrigendum to Respiratory cancer risks associated with low-level nickel exposure: An integrated assessment based on animal, epidemiological, and mechanistic data [Regul Toxicol Pharmacol 37(2003) 173-190] Regul Toxicol and Pharmacol, 41, 92-93.

Oller, A. 2002. Respiratory carcinogenicity assessment of soluble nickel compounds. Environ. Health Perspectives, 110:Supplement 5, 841-844.

Yu, C.P., Hsieh, T.H., Oberdörster, G., Oller, A. 2001 Evaluation of the human nickel retention model with workplace data. Regulatory Toxicology and Pharmacology, 33: 165-72.

Oller, A.R., Costa, M., Oberdörster, G. 1997. Carcinogenicity assessment of selected nickel compounds. Toxicol. And Appl. Pharmacol., 143: 152-166.

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Agenda

Monday, February 15, 2010

11:15 **Registration**

11:30 **Lunch**

12:00 **Meeting Convenes**[1]
Welcome and Panel Introductions, Ms. Jacqueline Patterson, *TERA*
Workshop Process, Dr. Michael Dourson, Chair

12:15 **Introduction to the Nickel Ion Bioavailability Model**
Dr. Adriana Oller, NiPERA
Dr. Julie Goodman, Gradient Corporation

Clarifying Questions from the Panel

1:00 **Discussion of i*n vitro* and Experimental Animal Data**
Charge Question 1

2:30 **Discussion of Epidemiological Evidence**
Charge Question 2

4:00` **Discussion of Hypothesis**
Charge Questions 3-8

5:30 **Adjourn for Day**

6:30 **Informal Dinner at Hotel (meeting room off lobby restaurant)**

Tuesday, February 16, 2010

8:00 ` **Meeting Re-convenes**
Discussion of Hypothesis (continued)
Questions 3-8

10:00 **Panel Conclusions and Data Needs**
Question 9

11:45 **Evaluation and Next Steps**

12:00` **Workshop Adjourns (Box lunches provided)**

[1] The Chair will call a break mid-morning and mid-afternoon.

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Workshop Background

This workshop of scientific experts is meeting on February 15 and 16 in Cincinnati, Ohio to discuss a proposed hypothesis for lung tumor induction after inhalation exposure to various nickel substances. The workshop will provide a critical evaluation of the Ni ion bioavailability model for lung tumor induction, as outlined in a draft paper prepared by Goodman and coworkers (Gradient). The goal is to determine whether the model is biologically plausible and coherent and is supported by the available data. In particular, the workshop will facilitate discussions of this model compared to the "nickel ion theory." Relevant epidemiological, animal and *in vitro* data will be reviewed and discussed. Workshop participants will also identify areas of consensus and areas of disagreement regarding the ability of the different models to explain the preponderance of the available data (epidemiology, animal, *in vitro*), the strengths and weaknesses of existing data to support or refute the models, the overall weight of evidence assessment regarding the respective models, and their utility in aiding the hazard assessment and/or risk assessment of nickel substances. Suggestions for alternative models that better support the available data will also be considered. Participants will also be asked to identify data gaps and specific research studies that can be undertaken to validate or disprove the bioavailability model. The workshop will focus on the science issues and will not discuss or determine cancer classification for regulatory purposes.

Workshop participants are encouraged to speak their opinions freely and are expected to represent their own individual expert opinions, not that of their employers or other groups with whom they are associated or identified. Workshop participants will be identified by name and affiliation in the meeting publications, and individual and summary panel opinions will be included in the public meeting report, but specific opinions and comments will not be attributed to individual panel members. The meeting is open to all interested persons to observe.

The workshop has been organized by Toxicology Excellence for Risk Assessment (*TERA)* with the Nickel Producers Environmental Research Association (NiPERA), which is providing funding. *TERA* is an independent non-profit organization with a mission to protect public health through the best use of toxicity and exposure information in the development of human health risk assessments. *TERA* has organized and conducted peer review and consultation meetings and workshops for private and public sponsors since 1996 (see www.tera.org/peer for information about the program and reports from meetings). NiPERA, Inc. is a not for profit organization and an independently incorporated division of the Nickel Institute (see www.nipera.org).

Inclusion of knowledgeable experts with a broad range of perspectives is key to the success of the workshop. NiPERA and the Nickel Institute have supported research on nickel toxicity issues by some of the world's leading experts in nickel. Individuals who have been supported by, or have financial ties to, NiPERA, the Nickel Institute (or other nickel interests) have not been excluded from this panel, and several of the panel members have received support from NiPERA in the past. These relationships are disclosed in the biographical sketches below. In addition, *TERA* as an organization has conducted work on nickel, including development of a toxicological assessment of nickel for the U.S. EPA, Health Canada, and the Metal Finishing Association of Southern California; a letter peer review of a nickel assessment document for the Texas Commission on Environmental Quality (2009); conduct or involvement in reviews of risk assessments for Port Colbourne, Sudbury and Flin Flon (Canada), and a private mining facility in South Africa, where nickel was a major chemical of concern (2001 - 2010); review and comment

on a draft journal article on carcinogenicity assessment of soluble nickel for NiPERA (2008); and, assistance to the Japan ICaRuS to develop a nickel cancer risk assessment for environmental exposure (2007).

The panel was sent the draft manuscript (Goodman et al., 2010) and a list of discussion questions in early January to ensure adequate time to carefully review the document and prepare for the meeting discussions. Prior to the meeting panel members provided preliminary comments on issues they thought should be considered. These were shared with the authors and rest of the panel to consider in preparation for the meeting. As these comments were preliminary and panelists may change their opinion upon further review and discussion, they will not be distributed further or made part of the official meeting record.

TERA will draft a meeting report that briefly summarizes the panel's discussions and recommendations. The meeting report will serve as a record of the workshop and assist in manuscript revision and/or identification of future research. The report will be reviewed by the panel members for accuracy before it is finalized.

Discussion Questions

In vitro and Experimental Animal Data

1. Do the available *in vitro* and *in vivo* data support the conclusions of Goodman et al. (2010) regarding:
    a. carcinogenicity of the various forms in animals
    b. respiratory toxicity
    c. clearance
    d. cellular uptake (ion transport, phagocytosis) and intracellular dissolution
    e. transport to the nucleus

    Are there other available data (either supportive or contrary) relevant to the above and is there potential for alternative interpretations of the data regarding nickel carcinogenicity in animals?

Epidemiology Evidence

2. Goodman et al. (2010) conclude that the epidemiological data support both the nickel ion hypothesis and the bioavailability hypothesis. They conclude that the epidemiological data are not sufficiently robust for determining which hypothesis is most appropriate, but are consistent with the nickel ion bioavailability hypothesis. Do the available epidemiology data support this conclusion? Could the data support a different conclusion? Do the data support one hypothesis over another? Should other available data be discussed?

Overall Review of Hypothesis

3. How strong is the overall integration of the *in vitro* data, and human and experimental animal data (by relevant routes of exposure) to support the bioavailability hypothesis. What evidence is counter to this proposed hypothesis?

4. Are there other hypotheses that might explain the data better than the bioavailability model (e.g., a tumor-promoting mechanism that does not depend on direct nuclear interactions; or evocation of tumors based on lung inflammation, the nickel ion hypothesis, the amount of nickel inhaled or retained in the lung, or something else)?

5. In focusing on nickel reaching the nucleus, the authors suggest that, even if the effects of the nickel ion in the nucleus are assumed to be via genotoxicity, a "practical threshold" for initiation of carcinogenicity exists. Please comment on this assertion.

6. The bioavailability hypothesis focused on lung cancer. ICNCM (1990) also found that several forms of nickel were associated with increased nasal cancer risk in the epidemiology studies, but nasal cancer was not reported in any of the experimental animal studies with inhaled nickel. Should the bioavailablity hypothesis (or other hypotheses addressing nickel carcinogenicity) consider other tumor types in addition to the lung?

7. Can an overall weight of evidence conclusion be made at this time? If not, what further analyses might help?

8. Are there other issues or questions that should be discussed relative to the nickel ion bioavailability hypothesis and its relevance to understanding the potential for carcinogenicity from nickel exposure?

Data Needs

9. Data needs are identified and discussed in the manuscript. Should additional data needs be added or deleted? Please rank the data needs according to which are essential to identify the determinants of nickel carcinogenicity and explain the differences observed among the various forms.

Appendix B: Presentation Slides

Workshop on Nickel Ion Bioavailability

February 15-16, 2010

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The Nickel Ion Bioavailability Model for Respiratory Tumor Induction
by Nickel-Containing Substances

Presentation I
Dr. Julie E. Goodman

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The Nickel Ion Bioavailability Model for Respiratory Tumor Induction by Nickel-Containing Substances

Julie E. Goodman, Ph.D., DABT

Nickel Ion Bioavailability Workshop
Northern Kentucky University
METS Center
February 15, 2010

Hypotheses

- Nickel Ion Bioavailability Model
  - A nickel-containing substance must release nickel ions that then become bioavailable at the nucleus of epithelial respiratory cells for the substance to be carcinogenic.
  - The carcinogenic potency of the substance is proportional to the degree to which the nickel ions are bioavailable at that site.
- Nickel Ion Theory
  - The nickel ion is carcinogenic, and if it can be released from a nickel-containing substance, then that substance should be considered carcinogenic.
  - One could extrapolate that water-soluble nickel compounds are the most potent carcinogens.

Carcinogenicity

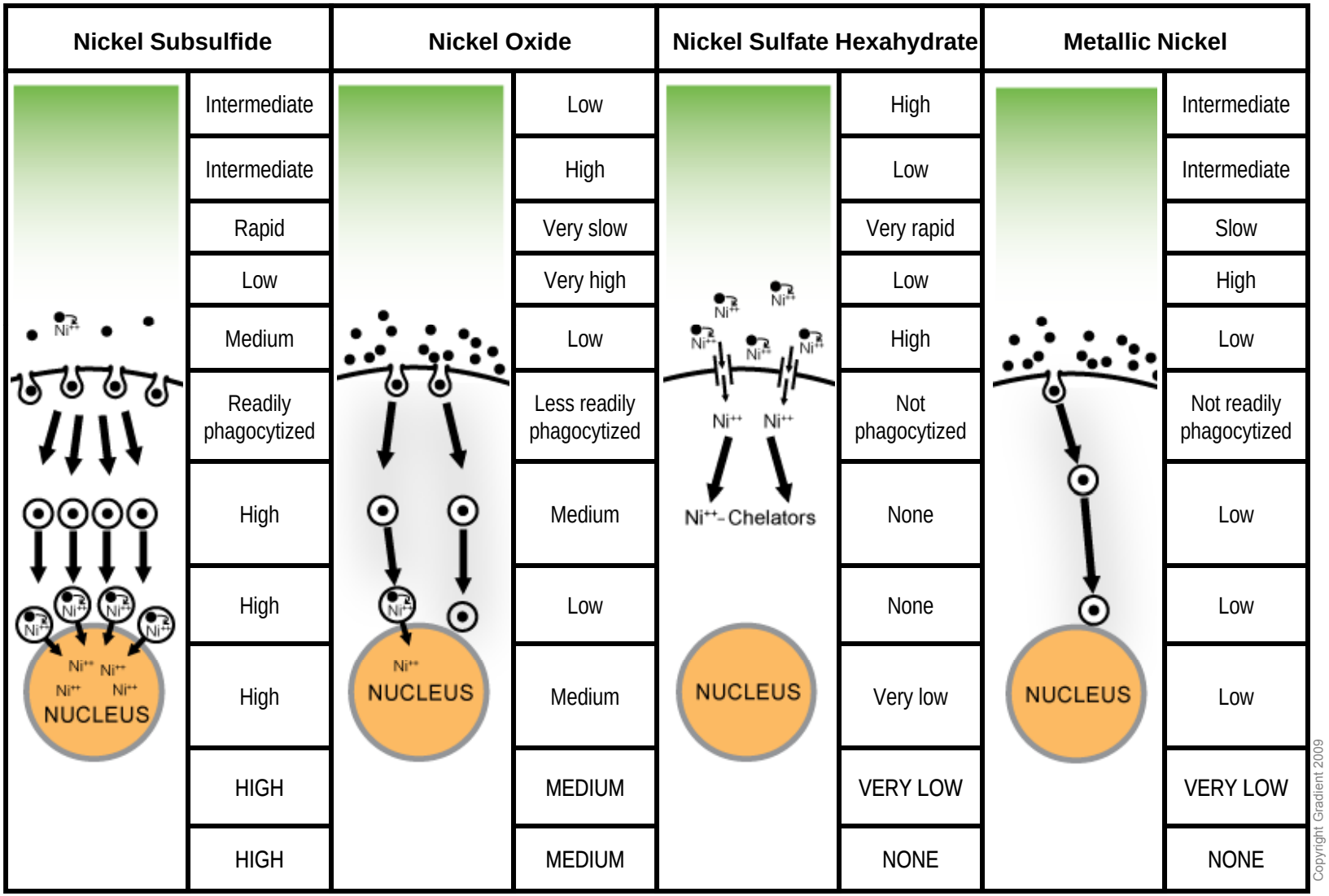
- Nickel Ion Bioavailability Model
  - Respiratory toxicity
  - Clearance
  - Extracellular dissolution
  - Intracellular uptake and dissolution
  - Sulfidic > oxidic >> water-soluble = metallic nickel
- Nickel Ion Theory
  - Solubility
  - Water-soluble > sulfidic = metallic > oxidic nickel

Animal Nickel Inhalation Studies

Nickel Form	Animal Species	Increased Incidence of Respiratory Tumors	Reference
Insoluble nickel subsulfide	Rats	Lung adenomas and carcinomas	NTP, 1996a
	Mice	No	
Insoluble nickel oxide	Rats	Lung adenomas and carcinomas	NTP, 1996b
	Mice	Lung adenomas and carcinomas	
Soluble nickel sulfate hexahydrate	Rats	No	NTP, 1996c
	Mice	No	
Metallic nickel	Rats	No	Oller *et al*., 2008

National Toxicology Program (NTP). 1996a. USDHHS. NTP TR 453. NIH Publication No. 96-3369
NTP. 1996b. USDHHS. NTP TR 451. NIH Publication No. 96-3367
NTP. 1996c. USDHHS. NTP TR 454. NIH Publication No. 96-3370
Oller, AR; Kirkpatrick, DT; Radovsky, A; Bates, HK. 2008. Toxicol. Appl. Pharmacol. 233:262-275.

Nickel Ion Bioavailability Model



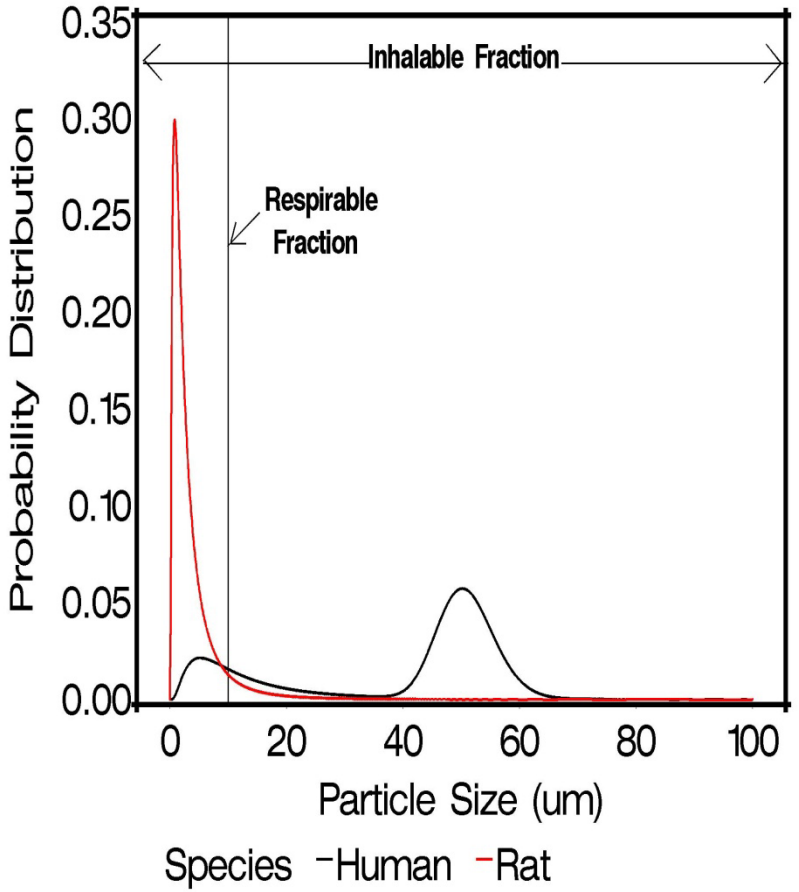
Epidemiology Analysis

- Reviewed key studies that address the association between sulfidic, oxidic, soluble, and metallic nickel and lung cancer risk
- Compared workplace sulfidic and oxidic nickel exposures to the human equivalent concentrations (HECs) corresponding to the animal lowest observable adverse effects concentration (LOAEC)
  - Bioavailability model predicts increased lung cancer risk with exposures > LOAEC(HEC)
- Categorized soluble nickel equivalent values: < 0.1 mg Ni/m^3; 0.1 to ≤ 1 mg Ni/m^3; > 1 mg Ni/m^3
  - Nickel ion theory predicts higher risks with higher solubility

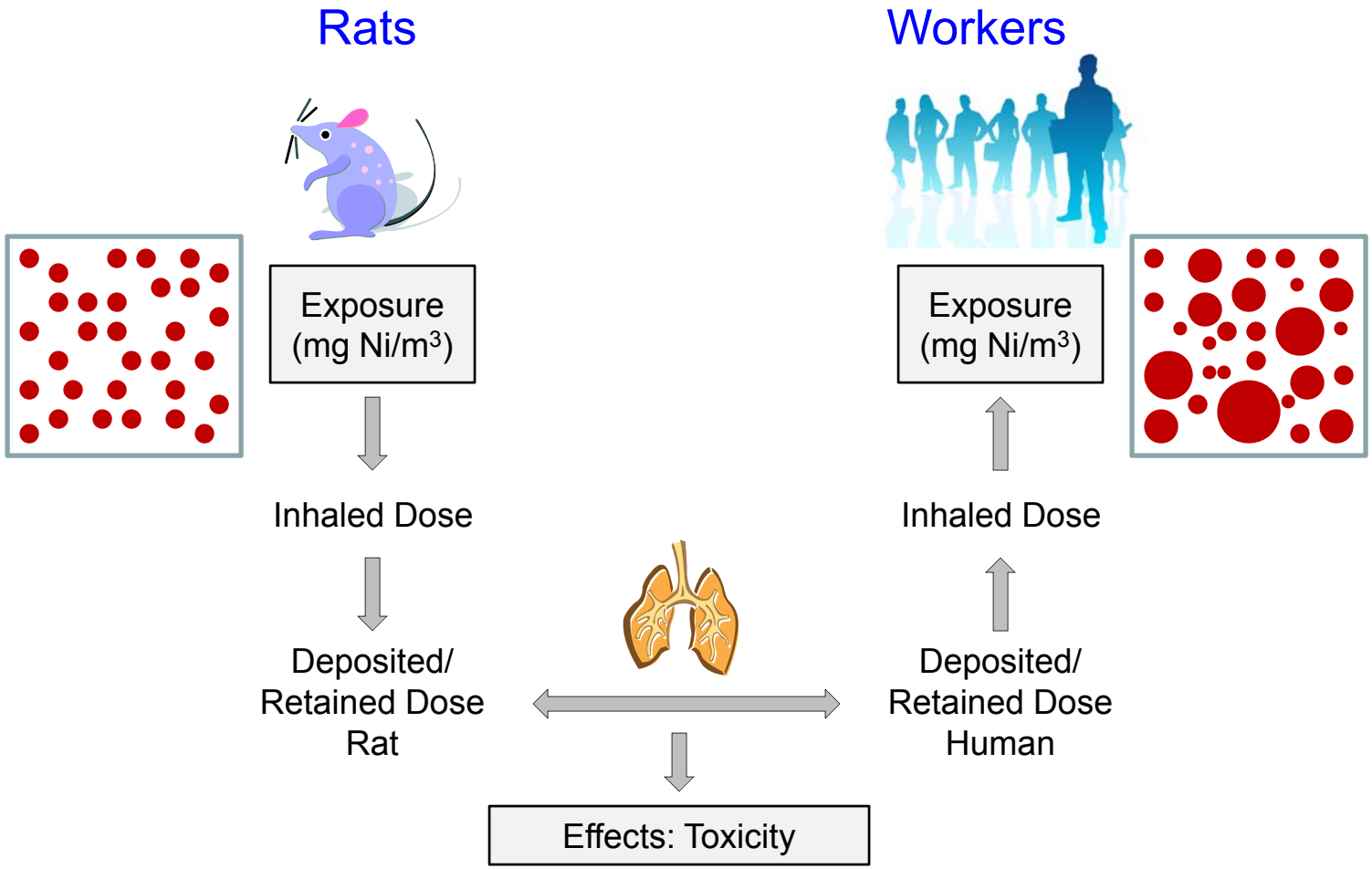
Particle Size Differences in Aerosols

- Rat or mice inhalation studies are conducted with homogeneous aerosols of small particle size (MMAD = 2 µm, GSD = 2)

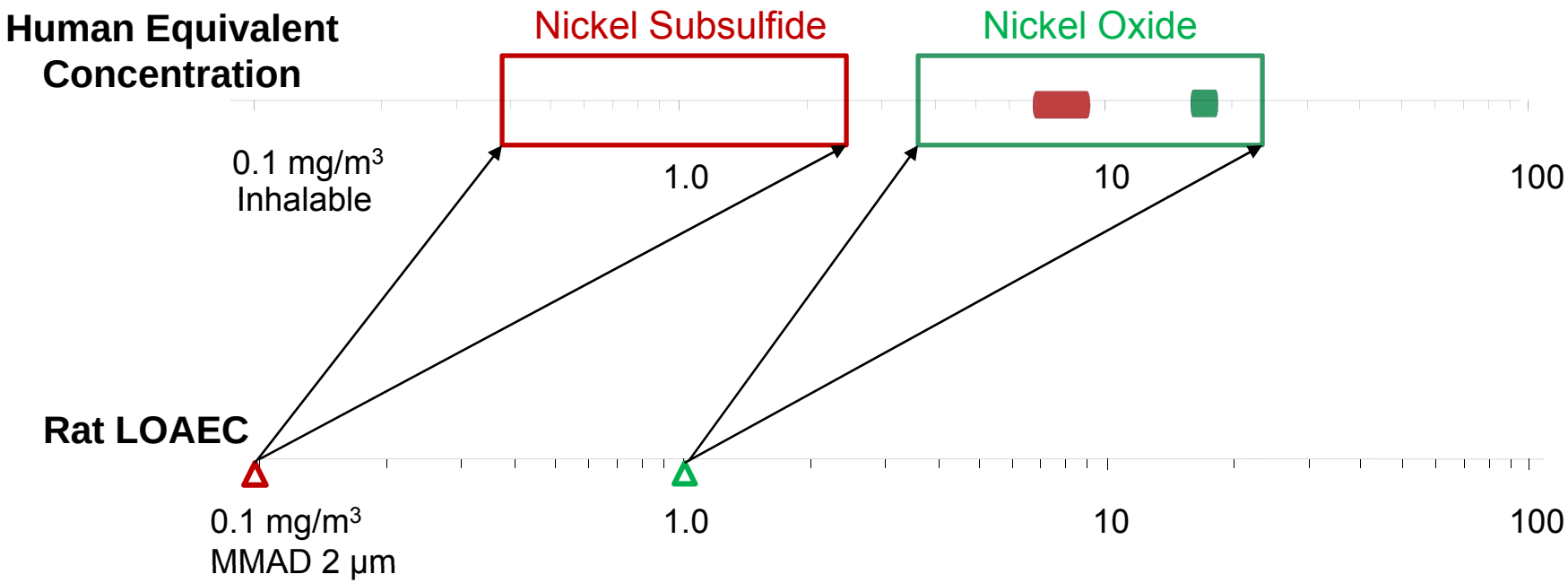
- Workplace nickel exposures are mostly to "inhalable" size particles (< 100 µm d_a)



Comparison Using a Dosimetric Approach



Rat Tumor LOAECs(HEC) and Human Occupational Exposures: Example



Range of Exposures to Sulfidic Nickel in Clydach Calcining Dep't

Range of Exposures to Oxidic Nickel in Clydach Calcining Dep't

Soluble Nickel Equivalents

Release of Ni^{++} (mg Ni^{++}/g sample) after 24 h at 37 ºC		
Sample	Synthetic Lung fluid	
	Alveolar	Interstitial
Nickel Subsulfide	6.7	26
Green Nickel Oxide	0.5	0.7
Nickel Chloride Hexahydrate	137	120
Nickel Metal Powder	2.3	1.6

Estimated based on the ratio of the solubility (*i.e.*, release of Ni^{++}) of each nickel species compared to water-soluble nickel

Soluble Equivalent in:

Alveolar Fluid: Soluble + (6.7/137)*Sulfidic + (0.5/137)*Oxidic + (2.3/137)*Metal

Interstitial Fluid: Soluble + (26/120)*Sulfidic + (0.7/120)*Oxidic + (1.6/120)*Metal

Epidemiology Analysis

Industry Sector/Cohort		Bioavailability Model		Nickel Ion Theory		Lung Cancer Risk
		Estimated Exposures		Soluble Nickel Equivalents		
		Sulfidic	Oxidic	Alveolar	Interstitial	
Refining Operations: High Insoluble & Water-Soluble Nickel Exposures	Linear Calcining Department, Mond/INCO Refinery, Clydach, Wales					↑↑
	Copper Plant, Mond/INCO Refinery, Clydach, Wales					↑↑
	Sinter Plant, Copper Cliff, Ontario, Canada					↑↑
	Leaching, Calcining, and Sintering, Port Colborne, Ontario, Canada					↑↑
	Roasting, Smelting, and Calcining, Falconbridge Nickel Refinery, Kristiansand [a]					↑↑
	Roasting, Smelting, and Calcining, Falconbridge Nickel Refinery, Kristiansand [b]					↑↑
	Huntington Alloys, Inc., West Virginia, US (before 1947)					–
Refining Operations: Low Insoluble & High Water-Soluble Nickel Exposures	Electrolysis, Falconbridge Nickel Refinery, Kristiansand, Norway [a]					↑↑
	Electrolysis, Falconbridge Nickel Refinery, Kristiansand, Norway [b]					↑↑
	Hydrometallurgy, Mond/INCO Refinery, Clydach, Wales					–
	Electrolysis, Port Colborne, Ontario, Canada					–
	Refinery, Outokumpu Oy, Harjavalta, Finland					↑↑
	Smelter, Outokumpu Oy, Harjavalta, Finland					↑↑
Refining Operations: High Metallic Nickel Exposures	Hydrometallurgy, Saskatchewan, Alberta, Canada					–
Sulfidic Ore Mining and Smelting: Low Insoluble & Water-Soluble Nickel Exposures	Mining, Milling, Smelting Operations, Falconbridge, Ontario, Canada					↑ [c]
	Mining, Milling, Smelting Operations, INCO, Ontario Canada					↑ [c]
Mining and Smelting of Lateritic Ores	Societe le Nickel Mining and Smelting Operations, New Caledonia					–
	Hanna Mining and Smelting Operations, Oregon, US					–
Alloy Manufacturing and Grinding	Henry Wiggin Alloy Company, Hereford, UK					–
	Huntington Alloys, Inc., West Virginia, US (after 1946)					–
	Nickel Alloy Workers from 13 Plants, US					↑/–
	Powder Metallurgy, Nickel Alloy Workers from 13 Plants, US					–
Barrier Manufacturing	Gaseous Diffusion Plant, Oak Ridge, Tennessee, US					–
Nickel Plating	Nickel Plating Factory, Birmingham, Midlands, UK					–

[a] *ICNCM (1990)*
[b] *Grimsrud et al. (2003)*
[c] *Risks likely attributable to smoking (Seilkop and Oller, 2003)*

Exposures
- *≤ rat tumor LOAEC(HEC)*
- *> rat tumor LOAEC(HEC)*

Soluble nickel equivalents
- *< 0.1 mg Ni/m³*
- *0.1 to ≤ 1 mg Ni/m³*
- *> 1 mg Ni/m³*

Statistically Significant Risk
- ↑ *> 100*
- ↑↑ *≥ 200*

Data Gaps

- Nickel-copper oxide animal bioassays
- Metallic nickel clearance
- Relative uptake of nickel-containing substances in rat and human lung cells
- Human data
- Physiologically-Based Pharmacokinetic (PBPK) studies (*e.g.,* Hack *et al.,* 2007)

Conclusions

- Animal and mode-of-action (MOA) data
  - Support nickel ion bioavailability model
  - Do not support nickel ion theory
- Epidemiology data
  - Consistent with both hypotheses
- A few data gaps remain
- Data available to date better support nickel ion bioavailability model

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The Nickel Ion Bioavailability Model for Respiratory Tumor Induction
by Nickel-Containing Substances

Presentation II
Dr. Harvey Clewell

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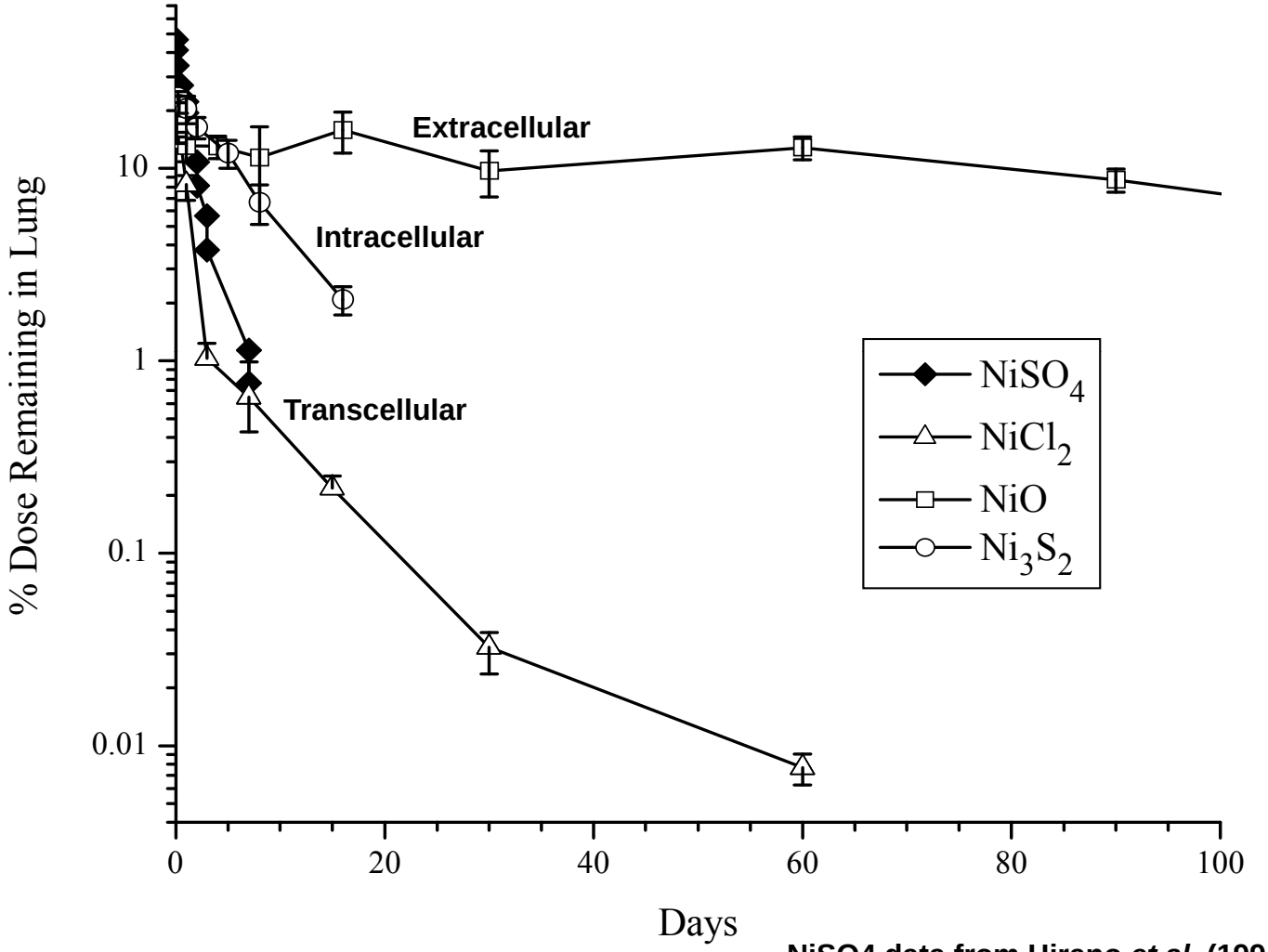
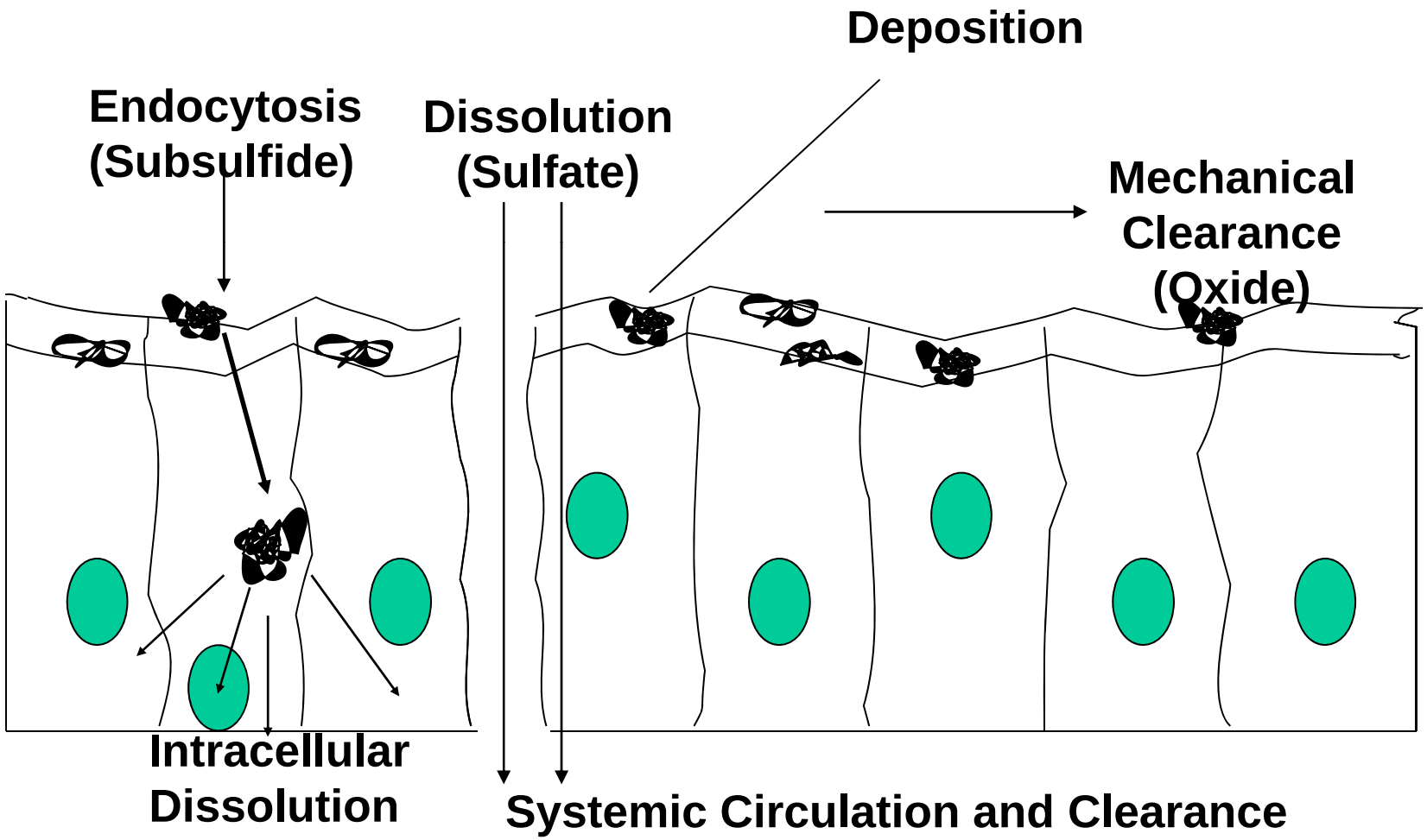
Lung Dosimetry of Nickel Compounds: In Vivo Clearance



NiSO4 data from Hirano *et al*. (1994);
NiCl2 data from English *et al*. (1981);
Ni2O3 and Ni3S2 data from Benson *et al*. (1994).

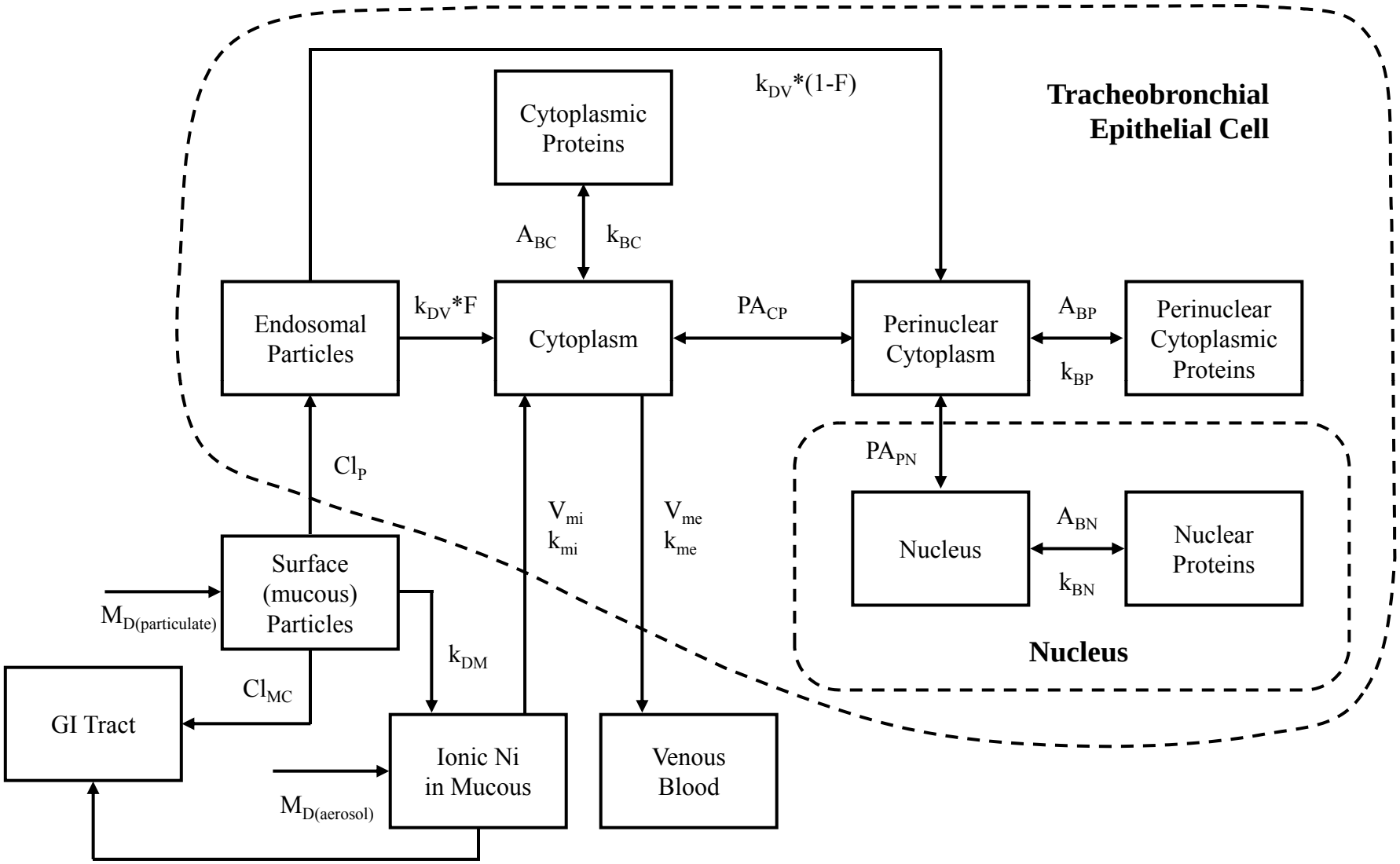
Dosimetry for Inhaled Nickel



Pharmacokinetic Model of the Intracellular Dosimetry of Inhaled Nickel

- A preliminary model of a lung epithelial cell was developed to describe the differences in the cellular uptake and intracellular kinetics of the different classes of nickel compounds
- Data available in from published studies with pneumocytes (soluble Ni) and CHO cells (Ni particles) were used to define the initial model parameters
- The resulting cellular dosimetry model is able to describe kinetic data on three forms of nickel (soluble, insoluble sulfide, and subsulfide)
- This preliminary model development effort has identified critical data gaps that could be filled by additional research
- The ultimate goal would be to integrate a refined cellular dosimetry model with models of lung deposition/clearance and systemic distribution/clearance

Biologically Based Model of Cellular Nickel Kinetics in the Tracheobronchial Epithelium (Hack et al. 2007)



M_D = Deposited dose = f(MMAD,conc)
Cl_{MC} = Mucocilliary clearance
V_{mi}, k_{mi} = Influx
V_{me}, k_{me} = Efflux

A_B, k_B = Binding (BC: cytoplasm, BN: nucleus, BP: perinuclear)
Cl_P = Endocytosis
PA_{PN} = Diffusion into nucleus (PN: nucleus to perinuclear, CP: cytotoplasm to perinuclear)
k_D = Dissolution rate (DM: mucous, DV: endosomes)

Parameter		Value	Reference
Maximum influx rate (µmol/hr/µm^2)	**$V_{mi}C$**	**10**	**Saito and Menzel (1986)**
Influx half-max. concentration (µmol/pL)	**k_{mi}**	**1**	**Saito and Menzel (1986)**
Maximum efflux rate (µmol/hr/µm^2)	**$V_{me}C$**	**0.001**	**Saito and Menzel (1986)**
Efflux half-max. concentration (µmol/pL)	**k_{me}**	**1**	**Saito and Menzel (1986)**
Extracellular Dissolution Rate (d^{-1})			
Crystalline NiS	**k_{DM}**	**0.00072**	**Costa and Heck (1984), Sunderman *et al.* (1987)**
Ni_3S_2	**k_{DM}**	**0.024**	
Endosomal Dissolution Rate (d^{-1})			
Crystalline NiS	**k_{DV}**	**0.3**	**Abbracchio *et al.* (1982)**
Ni_3S_2	**k_{DV}**	**0.36**	
Intracellular Diffusion Rate (µm^2/hr)			
Cytoplasm – Perinuclear	**$PA_{CP}C$**	**0.011**	**Abbracchio *et al.* (1982), Costa *et al.* (1981b)**
Perinuclear – Nucleus	**$PA_{PN}C$**	**1.5**	
Endocytosis (pL/hr/cell)			
Crystalline NiS	**Cl_pC**	**500**	**Costa *et al.* (1981b), Feren *et al.* (1992)**
Ni_3S_2	**Cl_pC**	**1000**	
Binding Maximum (µmol/pL)			
Cytoplasm	**$A_{BC}C$**	**0.1**	**Abbracchio *et al.* (1982), Costa *et al.* (1981b), Saito and Menzel (1986)**
Perinuclear	**$A_{BP}C$**	**0.1**	
Nuclear	**$A_{BN}C$**	**1**	
Binding Afinity (µmol/pL)			
Cytoplasm and perinuclear	**k_{BC}**	**1**	**Abbracchio *et al.* (1982), Costa *et al.* (1981b), Saito and Menzel (1986)**
Nuclear	**k_{BN}**	**1**	
Fraction Released from Endosome			
Crystalline NiS	**Frac**	**0.0375**	**Abbracchio *et al.* (1982), Evans *et al.* (1982)**
Ni_3S_2	**Frac**	**0.08**	

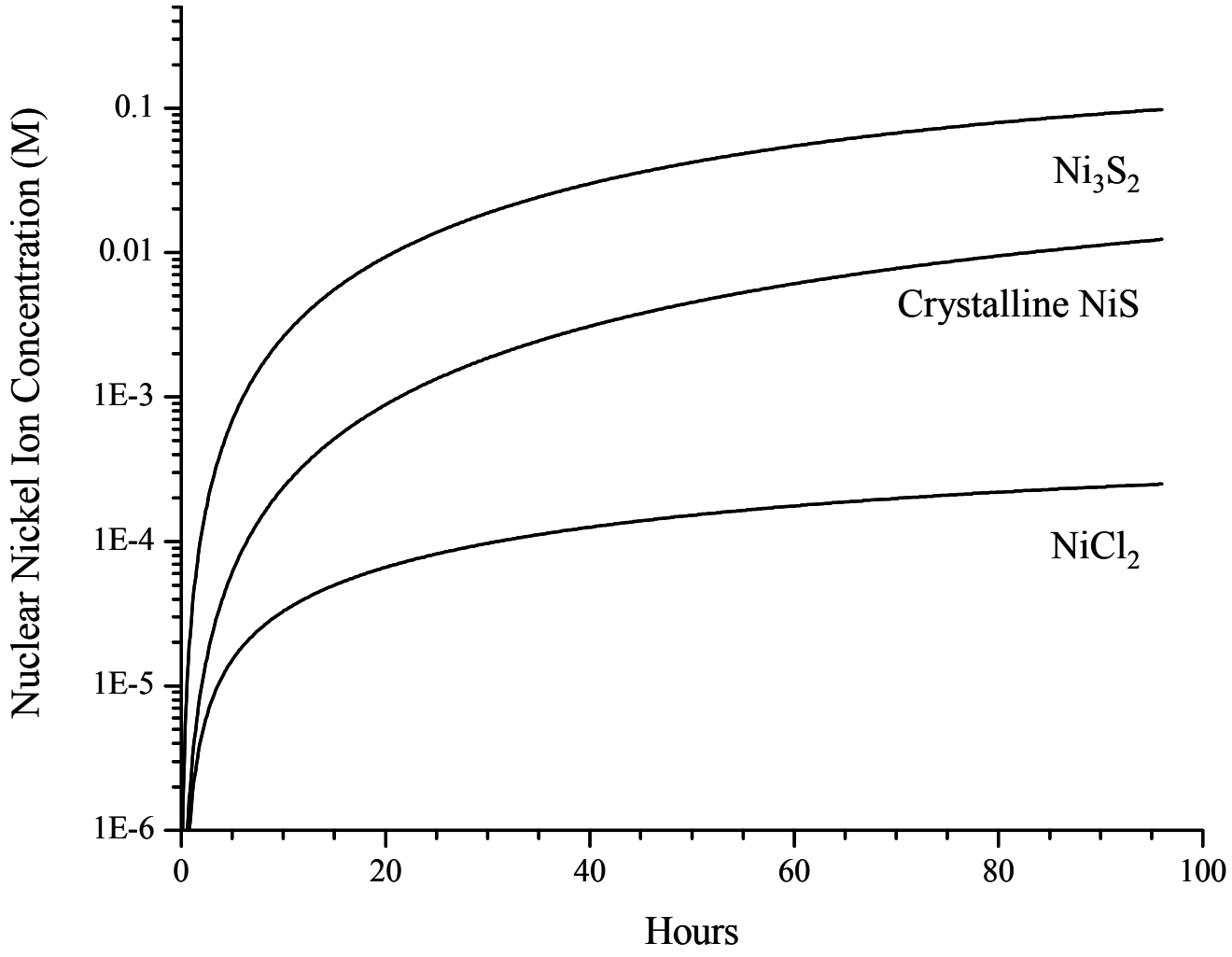
Comparison of Model Predictions and Observations of Costa *et al*. (1981b)

Compartment	Compound	Concentration	Time (hours)	Observed	Predicted
Nucleus (nmole/mg protein)	Ni_3S_2	5 µg/mL	24	14 ± 4.3	8.9
Nucleus (nmole/mg protein)	Ni_3S_2	10 µg/mL	24	17 ± 5.5	18
Whole cell (nmole/mg protein)	$NiCl_2$	2 µg/mL	24	0.78	1.2
Cytoplasm (nmole/mg protein)	$NiCl_2$	2 µg/mL	24	0.36	1.2
Nucleus (nmole/mg protein)	$NiCl_2$	2 µg/mL	24	0.020	0.040

Comparison of Model Predictions and Observations of Abbracchio *et al*. (1982)

Compartment	Compound	Concentration	Time (hours)	Observed	Predicted
Nucleus (nmole/mg protein)	$NiCl_2$	20 µg/mL	72	2.5	1.0
Nuclear fraction	$NiCl_2$	20 µg/mL	72	0.47	0.082
Cytoplasmic fraction	$NiCl_2$	20 µg/mL	72	0.17	0.92
Nucleus (nmole/mg protein)	Crystalline NiS	10 µg/mL	24	17 ± 5.8	4.9
Nuclear fraction	Crystalline NiS	11.25 µg/mL	96	0.28	0.29
Particulate fraction	Crystalline NiS	11.25 µg/mL	96	0.66	0.65
Cytoplasmic fraction	Crystalline NiS	11.25 µg/mL	96	0.063	0.057

Predictions of the preliminary cellular dosimetry model: Nickel ion delivered to nucleus of A-549 (human lung) cells incubated with 1x10-6 M NiCl2, crystalline NiS, or Ni3S2.



Pharmacokinetic Model of the Intracellular Dosimetry of Inhaled Nickel

- Data needed:
  - *In vitro* kinetic data
    - In tracheobronchial epithelial cells
    - Disposition over time
    - Both soluble and insoluble compounds
    - Multiple concentrations, timepoints
    - Consider “particokinetics” (Teeguarden)
  - *In vivo* validation data
    - Repeated inhalation exposure
    - Lung microdissection (C. Plopper, U.C. Davis)

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The Nickel Ion Bioavailability Model for Respiratory Tumor Induction
by Nickel-Containing Substances

Presentation III
Dr. Max Costa

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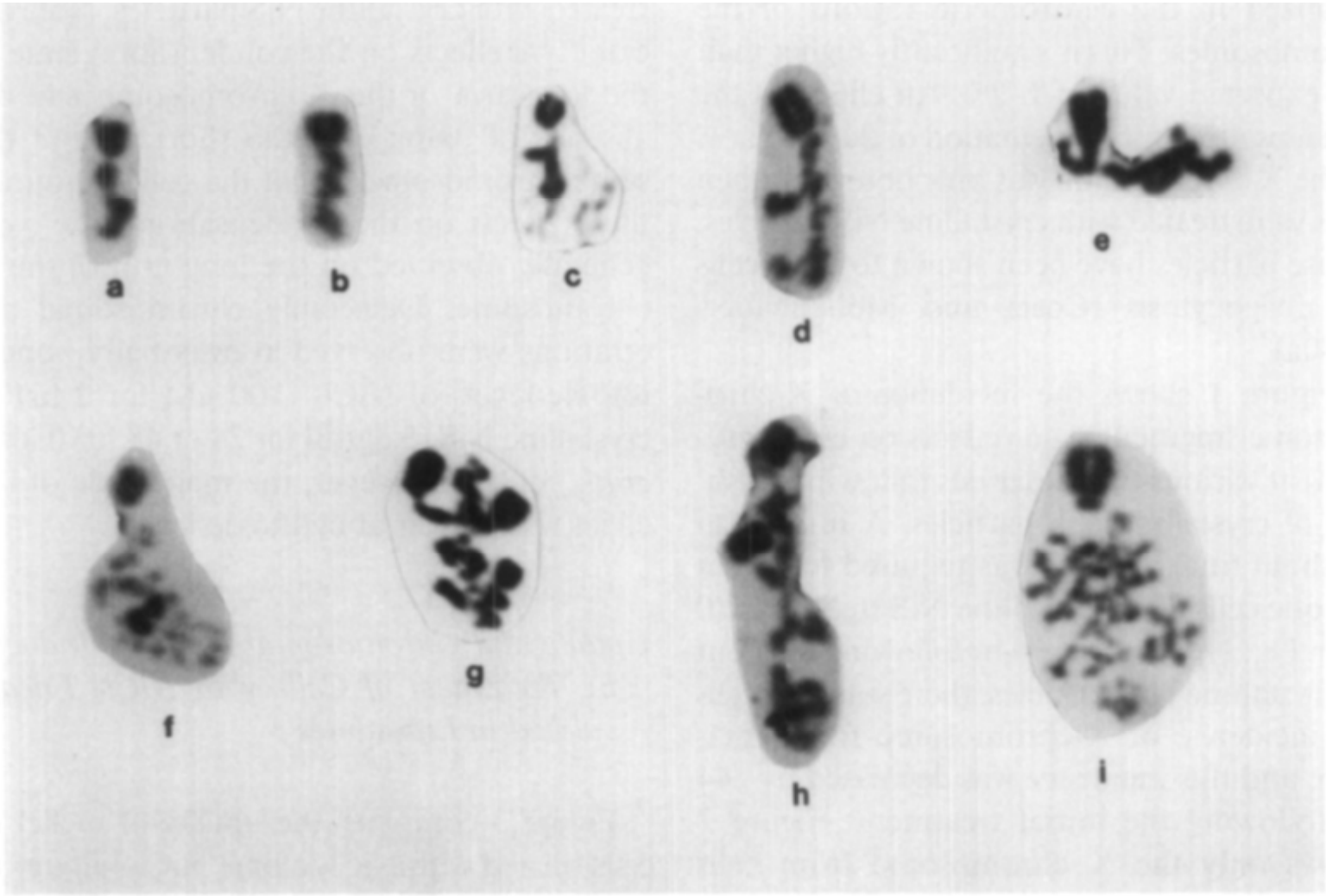

a
b
c
d
e
f
g
h
i

TABLE 1

EFFECT OF CRYSTALLINE NiS AND $NiCl_2$ ON CHROMOSOMAL ABERRATIONS

Chemical treatment	Treatment time (hr)	Cells with damage (%)	Types of aberrations[a]				Cells with multiple damage	Cells with X-chromosome fragmentation (%)
			G	B	E	D		
Crystalline NiS concentration (μg/ml)								
0	—	17.0[b]	8	7	0	0	0	0
5	24	20.0[b]	11	21	4	3	0	6
10	24	16.1[b]	16	16	2	1	1	3
20	24	40.3[b]	22	31	6	3	4	7
5	48	34.0[b]	19	31	8	9	6	7
10	48	36.6[b]	16	43	11	6	7	6
20	48	61.3[b]	38	49	33	12	9	12
$NiCl_2$ concentration (μM)								
0	2	10.2[c]	7	8	1	0	0	0
1	2	15.7[c]	8	12	1	0	0	0
10	2	25.7[c]	14	9	6	1	0	0
100	2	34.2[c]	18	17	8	7	0	0
500	2	58.7[c]	28	57	6	1	1	0
1000	2	42.5[c]	15	36	23	0	5	0

[a] G, gaps; B, breaks; E, exchanges, and D, dicentrics.

[b] Cells were treated in complete culture medium.

[c] Cells were treated in SCM.

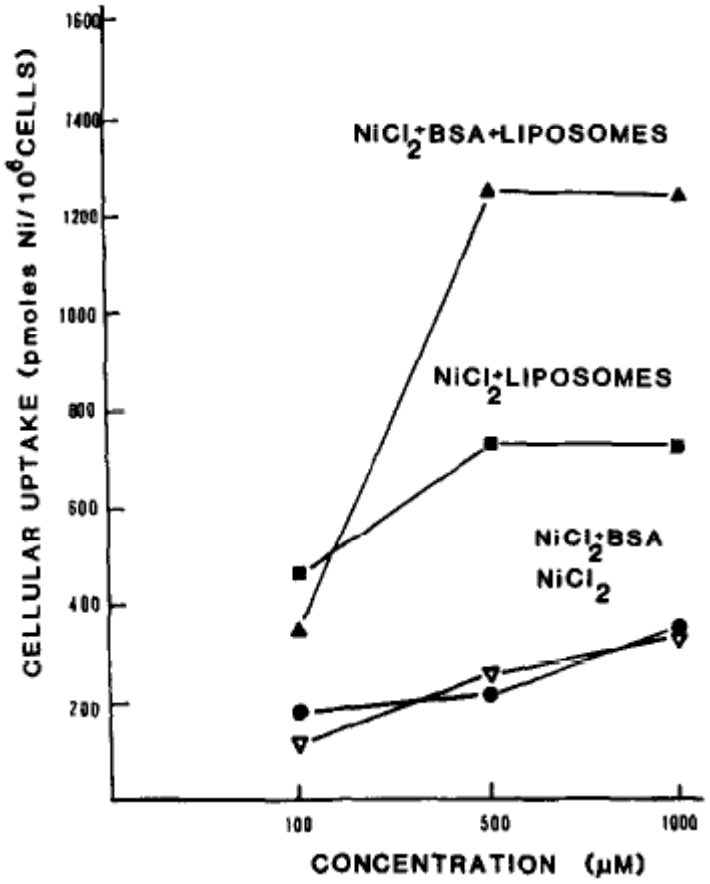

CELLULAR UPTAKE (pmoles Ni/10^6 CELLS)
1600
1400
1200
1000
800
600
400
200
NiCl2+BSA+LIPOSOMES
NiCl2+LIPOSOMES
NiCl2+BSA
NiCl2
100
500
1000
CONCENTRATION (μM)

TABLE 2

INDUCTION OF FRAGMENTATION OF THE X CHROMOSOME BY $NiCl_2$ ENCAPSULATED IN LIPOSOMES

Treatments	$NiCl_2$ concentration (μM)	Number of cells examined	Cells with X-chromosome fragmentation (%)
$NiCl_2$ + BSA[a]	100	315	0
	500	300	0
	1000	400	0
$NiCl_2$ + BSA + liposomes[a]	100	510	1.0
	500	542	3.5
	1000	866	3.0
$NiCl_2$ + BSA + liposomes[b]	100	526	2.0
	500	440	3.0
	1000	410	1.0
Liposomes + BSA (100 μg/ml)[a]	0	200	0
Liposomes + BSA (500 μg/ml)	0	200	0
Liposomes + BSA (1000 μg/ml)	0	150	0
$NiCl_2$ + Liposomes[a]	100	350	—
	500	300	2.0
	1000	450	1.0

Note. Chinese hamster ovary cells were treated with $NiCl_2$ in liposomes or $NiCl_2$–BSA in liposomes while being maintained in SGM for either 4 hr or in α-MEM for 24 hr. After the treatment, the cells were washed and allowed to recover for 24 hr before collecting mitotic cells.

[a] Cells were treated in SGM for 4 hr.

[b] Cells were treated in MEM for 24 hr.

Table 4

Uptake of $^{63}NiCl_2$ in CHO cells

CHO cells were exposed to $^{63}NiCl_2$ (2 μg/ml; 8 μCi/mg nickel) for 24 hr. Following this treatment, whole cells, nuclear fractions, and cytoplasmic fractions were isolated, and the radioactivity was determined in these fractions by liquid scintillation counting with a counting efficiency of 74%. Additionally, the radioactivity present in the 50% trichloroacetic acid-insoluble fraction was also determined. Samples were solubilized with 1.0 N NaOH (37° for 24 hr) prior to determining radioactivity. The base was neutralized in the scintillation fluor prior to counting using glacial acetic acid. Radioactivity was determined in 5 ml of 3A 70B liquid scintillation fluor obtained from Research Products International (Elk Grove Village, Ill.). Variation among replicate samples was <5%. The total number of determinations for each value was 3.

Cell fraction analyzed	Total (dpm/mg protein × 10^5)	Trichloroacetic acid insoluble (dpm/mg protein × 10^3)
Whole cell	8.1	3.1
Nuclei	2.1	9.6
Cytoplasm	4.2	1.1

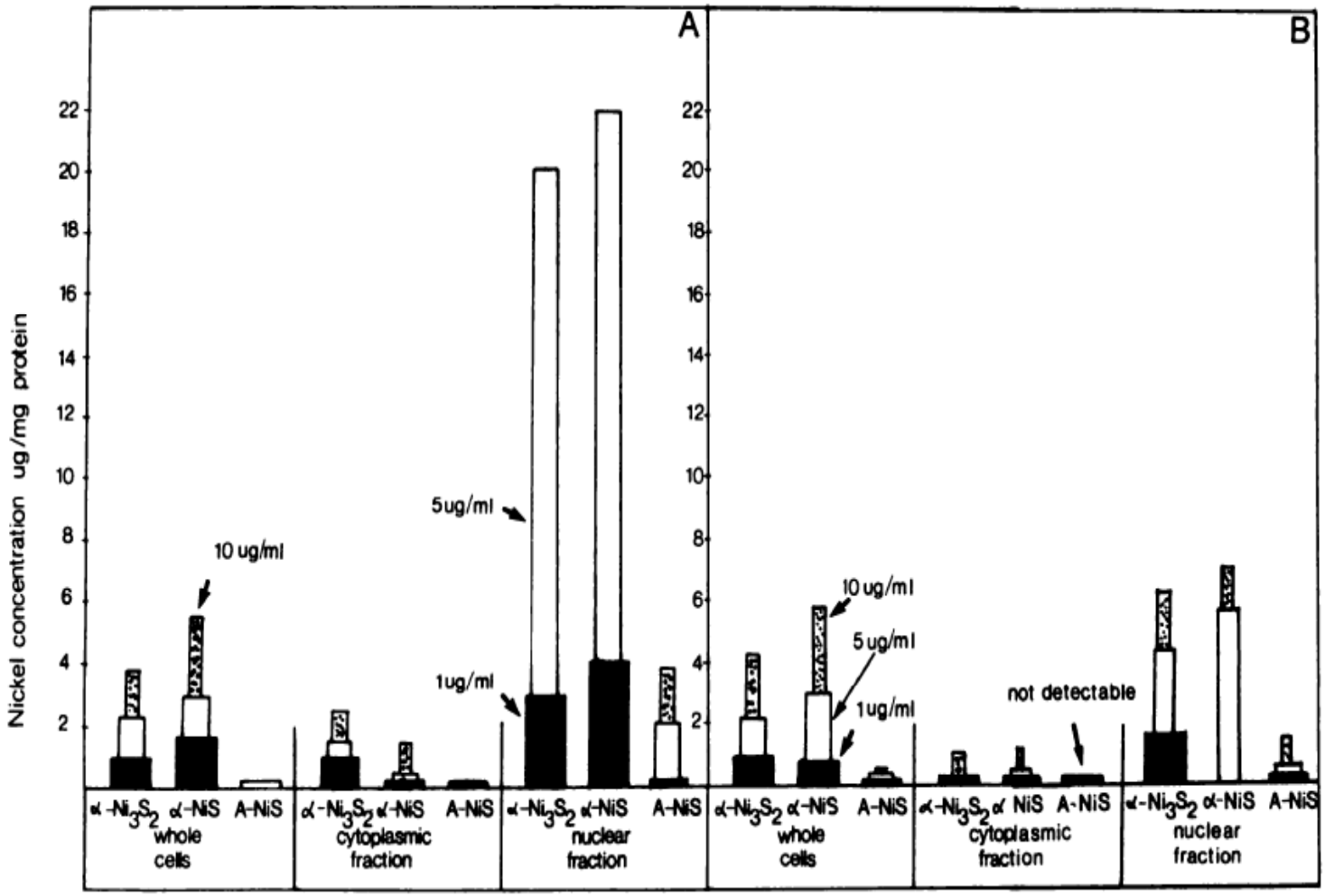
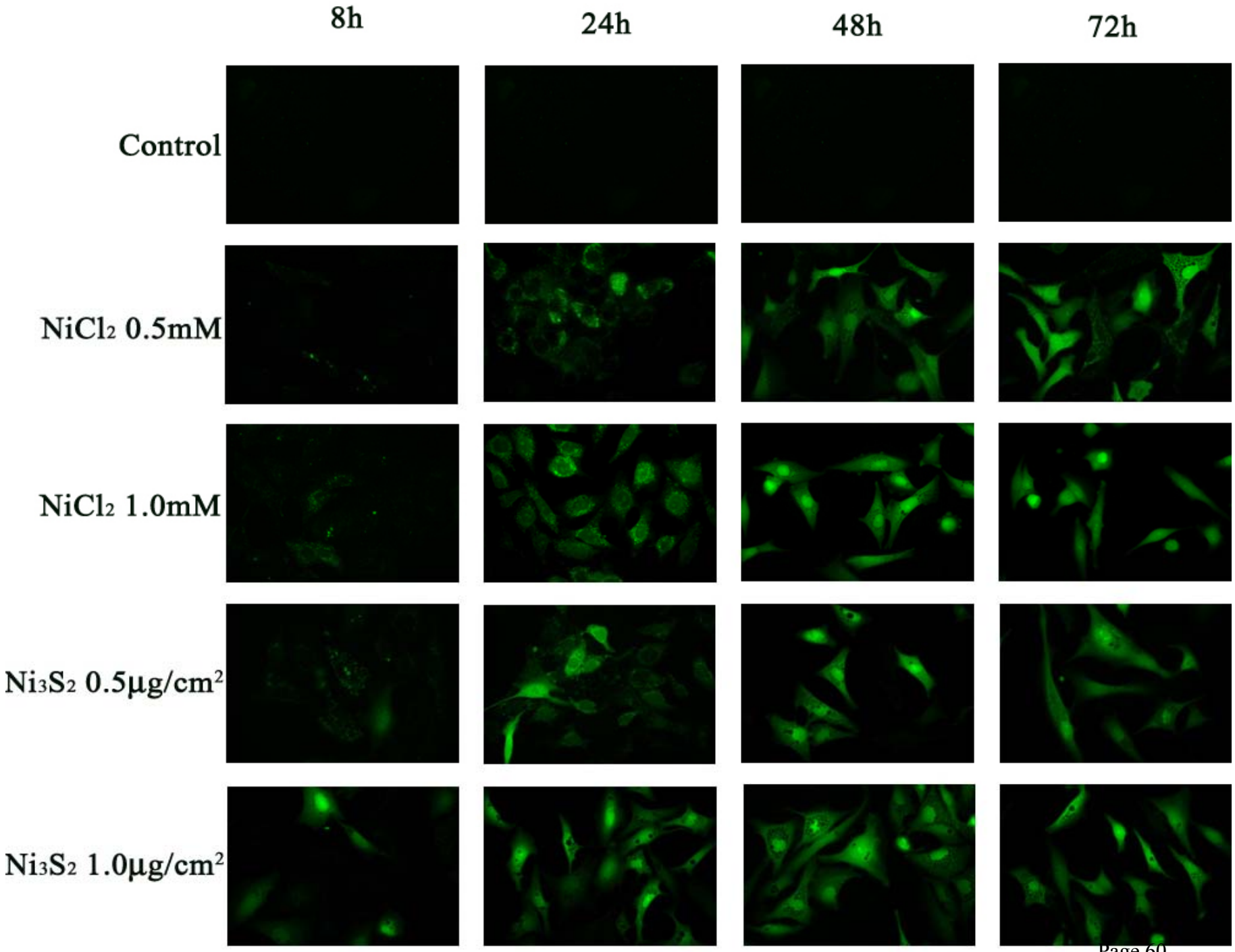


Chart 1. CHO cells were exposed for 24 hr (*A*) or 48 hr (*B*) to concentrations of 3 nickel compounds. *αNi$_3$S$_2$*, crystalline *αNi$_3$S$_2$*; *αNis*, crystalline *αNiS*; *A-NiS*, amorphous NiS. Exposures were performed in 100-mm Petri dishes using 10 ml of media. A 1-μg Ni_3S_2 exposure per ml therefore represents 0.72 μg(*ug*) nickel per ml or 0.09 μg nickel per sq cm of surface area. One μg of amorphous NiS or crystalline NiS represents 0.64 μg nickel per ml or 0.08 μg nickel per sq cm of surface area. Following these treatments, cells were washed twice with Puck's Saline A and dislodged from the monolayer by trypsin treatment. Suspensions of whole cells, cytoplasmic fractions, or nuclear fractions prepared as described under "Methods" were quantitatively analyzed for nickel concentration by X-ray fluorescence. Various amounts of nickel ranging from 20 to 100% were insoluble in 50% trichloroacetic acid in each of these fractions. Generally, in the cytoplasmic fraction, about 20% of the nickel was insoluble in 50% trichloroacetic acid, while in the nucleus and whole cells about 50% of the nickel was insoluble in trichloroacetic acid. Protein concentration was determined using the commercially available Bio-Rad protein assay (Coomassie blue reaction). Protein was concentrated by trichloroacetic acid precipitation. Similar results were obtained in 2 additional experiments. To compare the amount of protein present in cytoplasm and nuclei from CHO cells, we averaged a number of values from several preparations. Nuclei contain approximately 10% of the protein in the cell, while the remaining 90% is cytoplasmic.

Potential Intracellular Concentration of a Phagocytized Crystalline NiS Particle[a]

Mean particle diameter used in calculation (μm)	Approximate NiS cellular concentration[b] (M)
1.45	0.25
4.00	4.75

[a]Cell volume was determined in CHO cells with a Coulter counter-particle size analyzer and log range expander.
[b]Cell volume, 393.5, μm^3; density of NiS, 5.5g/cm^3; particles assumed spherical.


8h
24h
48h
72h
Control
NiCl2 0.5mM
NiCl2 1.0mM
Ni3S2 0.5μg/cm2
Ni3S2 1.0μg/cm2

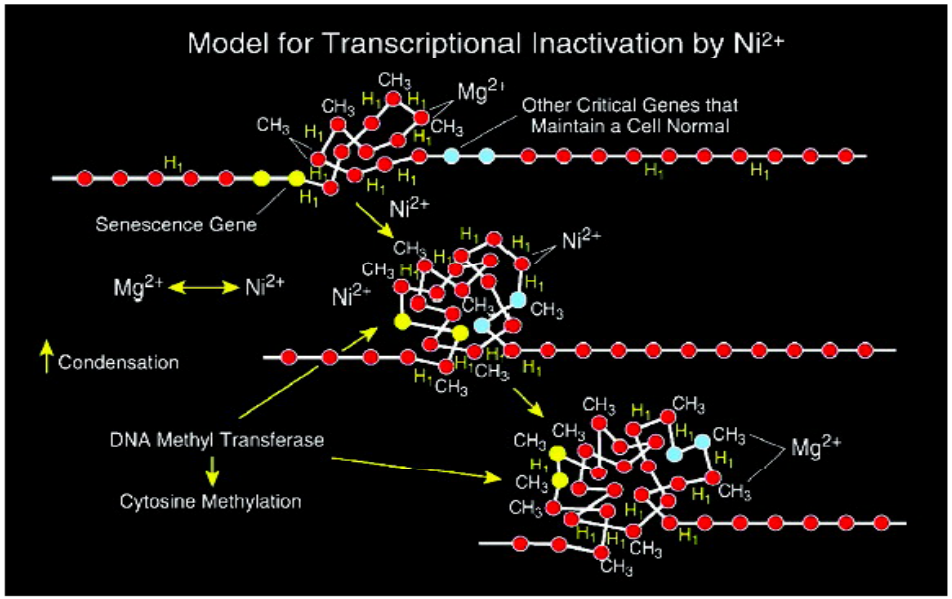
BIOCHEMISTRY
including biophysical chemistry & molecular biology

Article

Heterochromatinization as a Potential Mechanism of Nickel-Induced Carcinogenesis

Thomas P. Ellen, Thomas Kluz, Mark E. Harder, Judy Xiong, and Max Costa

Biochemistry. **Article ASAP** • DOI: 10.1021/bi900246h • Publication Date (Web): 01 April 2009



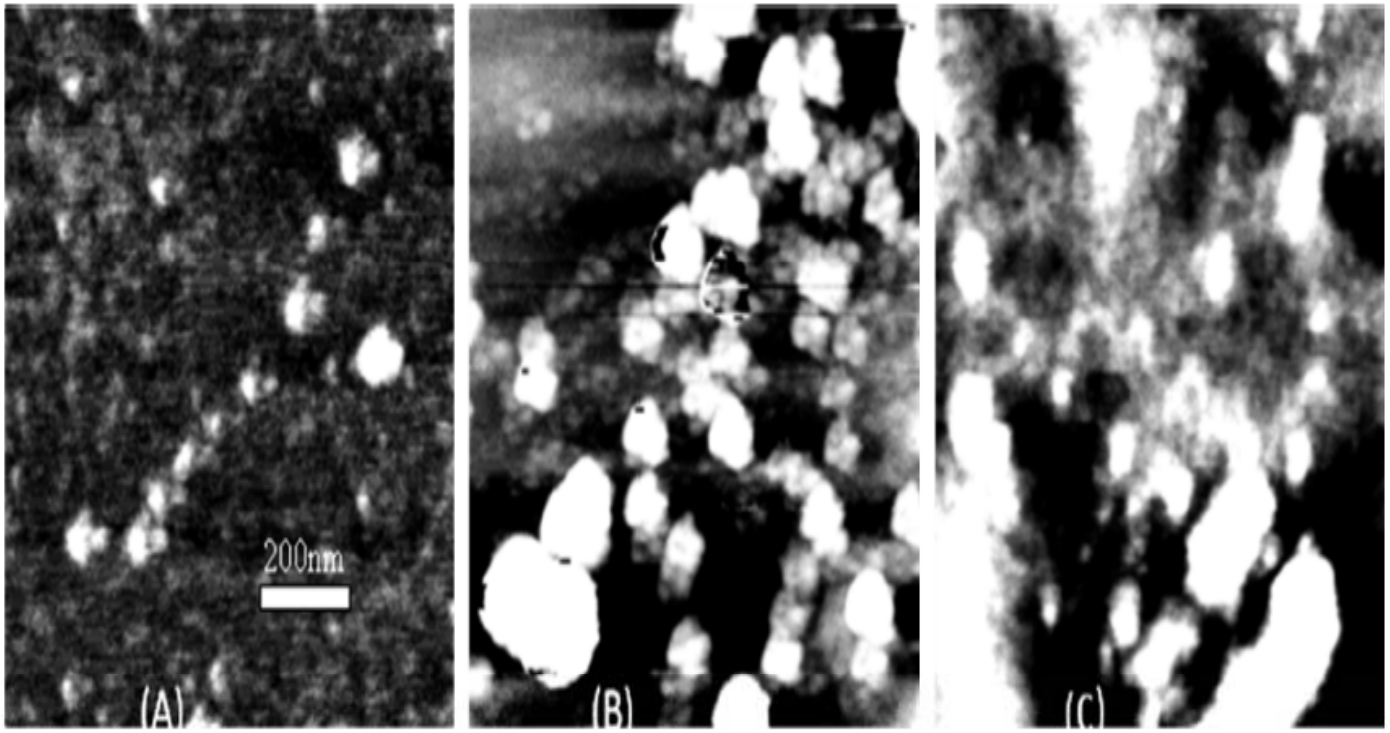


FIGURE 4: Atomic force microscopy (AFM) images of unfixed dodecanucleosomal fibers in TE buffer [10 mM Tris-HCl (pH 8.0) and 0.1 mM EDTA]: (A) no divalent cation, (B) treated with 1.0 mM Mg^{2+}, and (C) treated with 1.0 mM Ni^{2+}. For either divalent ion, 1.0 mM was the concentration at which the greatest, most significant and dramatic effects were observed.

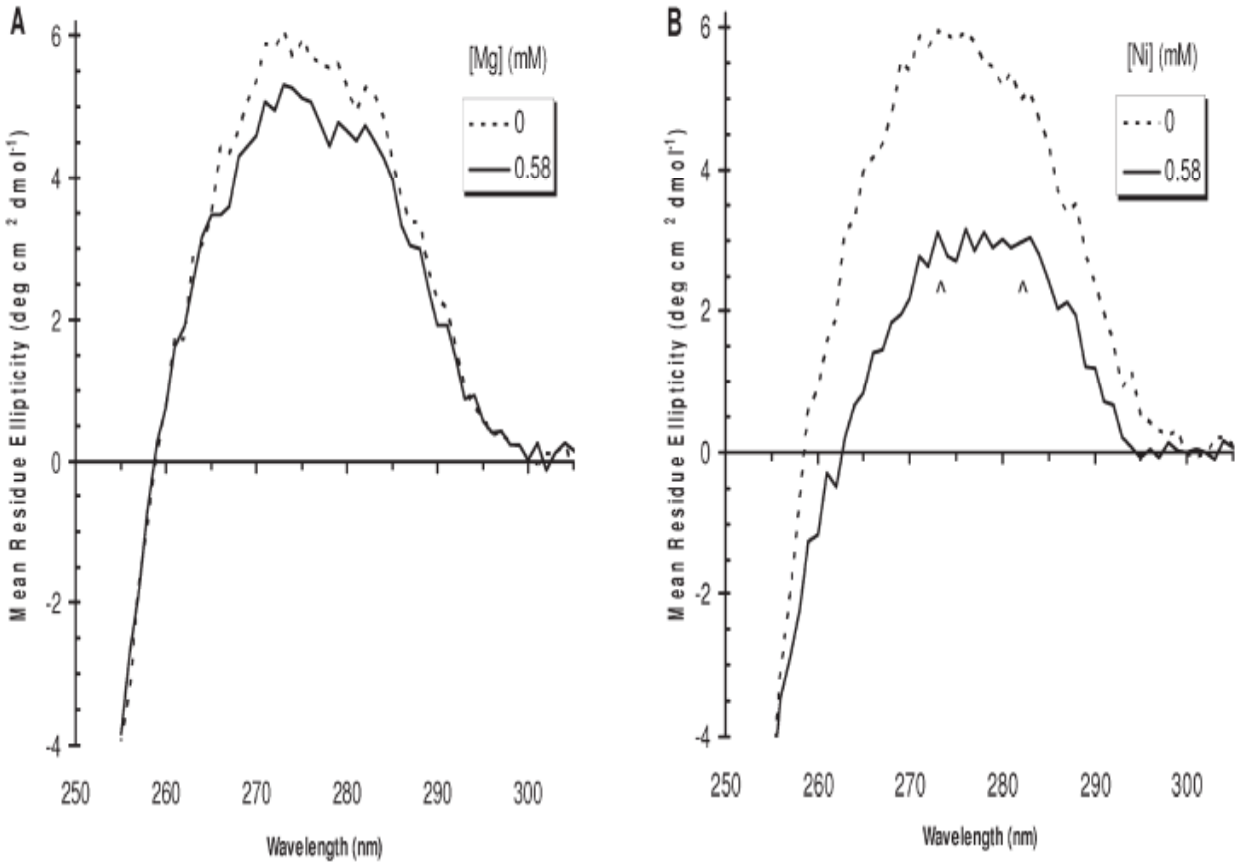


FIGURE 3: CD spectral difference for dodecanucleosomes in the absence or presence of divalent cations. Ions reduce the peak signal at 272 nm. The dashed lines are the CD spectra in the absence and solid lines those in the presence of divalent cation, at the indicated concentrations. Arrowheads in panel B show the two closely spaced peaks at 272 and 282 nm.

Table I. Evaluation of DCF in the cytosol and nuclei of CHO cells

	Treatment time (h)	Fluorescence intensity[a]		% fluorescence nuclei/cytosol
		Nuclei	Cytosol	
Control	6	13.9 ± 2.1	660.6 ± 83.4	2.1
$NiCl_2$ (2 mM)	6	16.9 ± 1.7	950.3 ± 130.2	1.8
Ni_3S_2 (20 $\mu g/cm^2$)	6	13.7 ± 2.1	1006.0 ± 119.9[b]	1.4
Control	18	17.1 ± 4.2	837.1 ± 152.8	2.0
$NiCl_2$ (2 mM)	18	20.9 ± 5.1	2050.3 ± 130.2[b]	1.0
Ni_3S_2 (0.5 $\mu g/cm^2$)	18	13.1 ± 3.6	412.9 ± 98.0[b]	3.2
Ni_3S_2 (2 $\mu g/cm^2$)	18	62.2 ± 14.2[b]	374.7 ± 70.8[b]	16.6

[a]CHO cells were treated with $NiCl_2$ for 6 or 18 h. The fluorescence intensity of DCF per cytosol or nuclear fraction derived from 10^6 cells/ml is presented as the actual meter reading divided by a factor of 100. The data shown are duplicate determinations from three independent experiments expressed as the mean ± SE.

[b]Significantly different from control, $P < 0.05$ by t-test.

The Nickel Ion Bioavailability Model for Respiratory Tumor Induction
by Nickel-Containing Substances

Presentation IV
Dr. Andrea Hartwig

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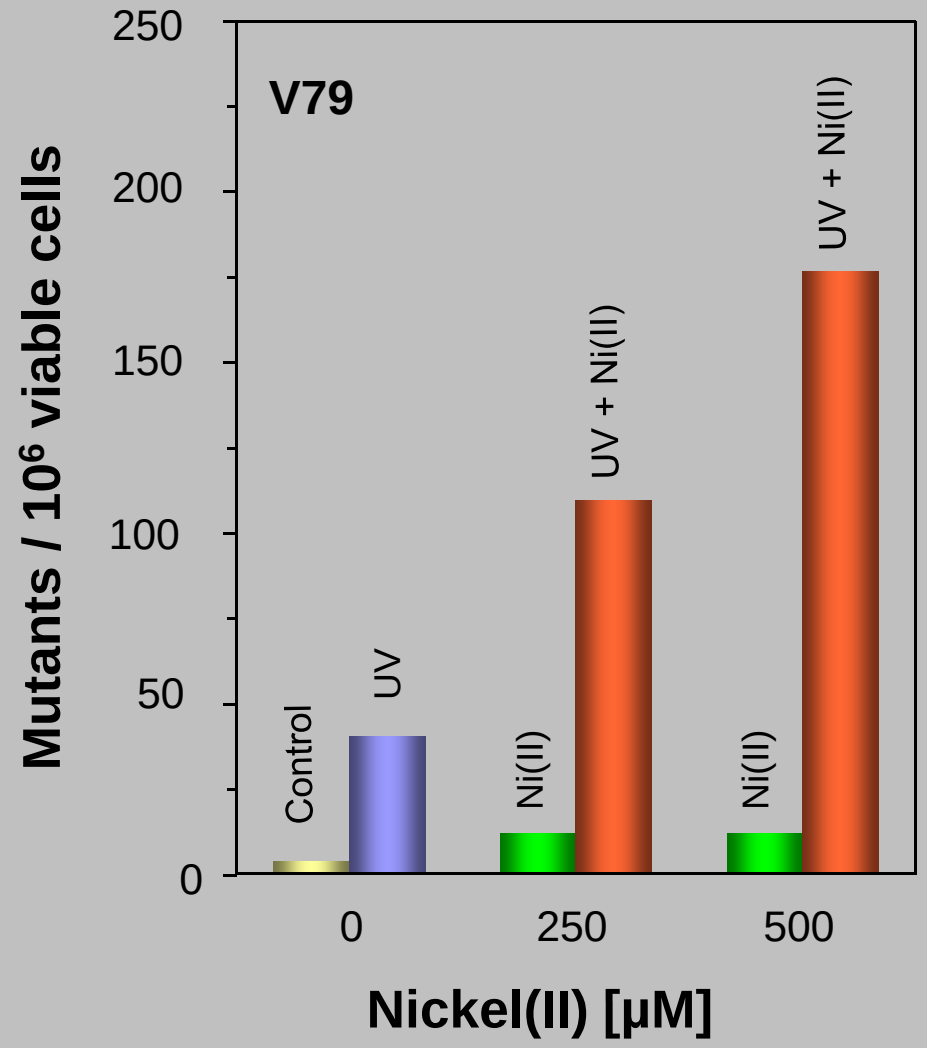
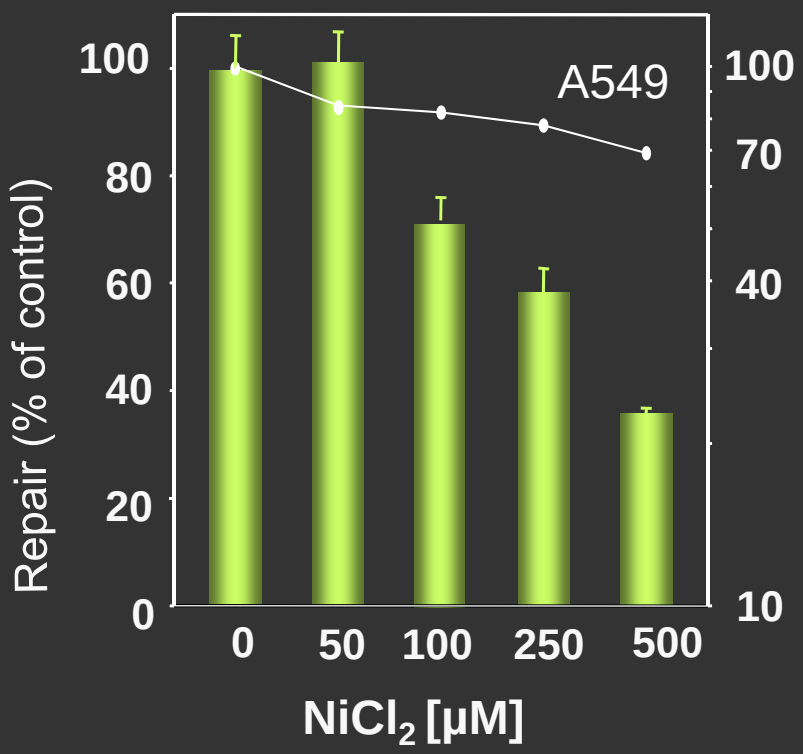
Mutagenicity and comutagenicity of nickel(II)

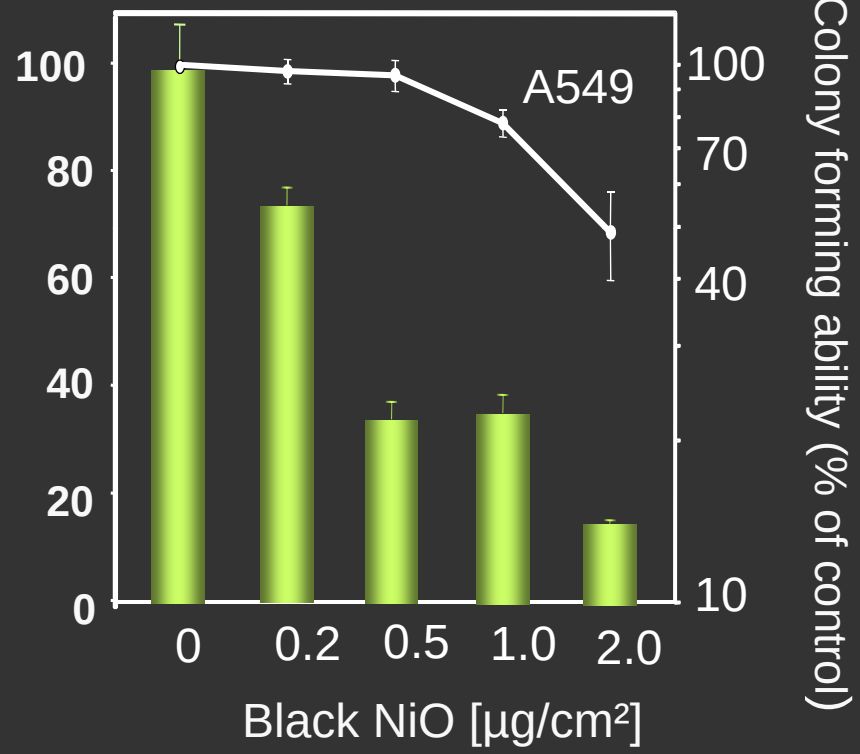


Hartwig and Beyersmann, 1989, Mutation Res. **217**, 65 - 73

Effect of nickel compounds on the repair of BPDE-induced DNA adducts



50 nM (+)-*anti*-BPDE, 6 h postincubation

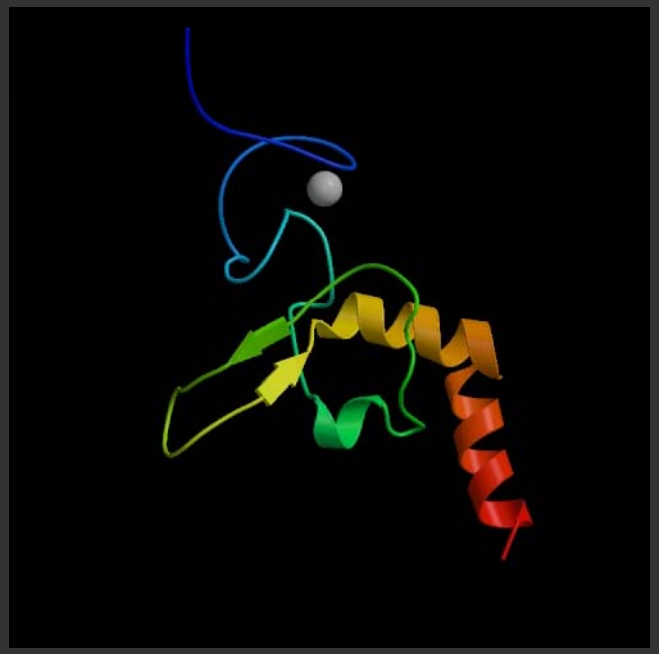


50 nM (+)-*anti*-BPDE, 8 h postincubation

Schwerdtle, T., A. Seidel and **A. Hartwig** (2002) *Carcinogenesis*, **23**, 47 – 53.

XPA protein and its zinc finger structure

- absolutely required for nucleotide excision repair (bulky DNA lesions, e.g., UV)
- defect in Xeroderma pigmentosum group A
- involved in assembly of DNA damage recognition/incision complex
- zinc finger structure (C_4-type)

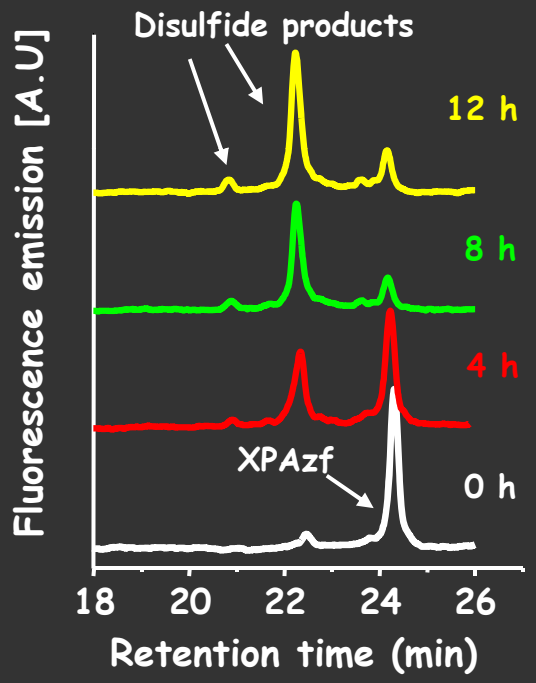


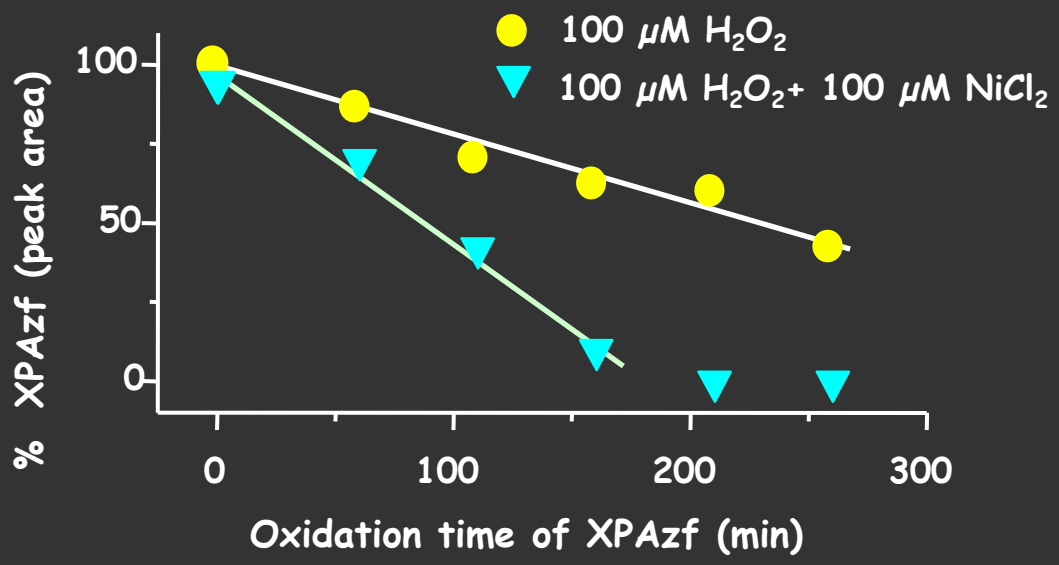
Effects of nickel and cadmium on XPAzf

Nickel:

→ **structural alterations** of the zinc finger domain

→ **increased sensitivity towards oxidation** (Bal et al., Chem Res Tox 2004)



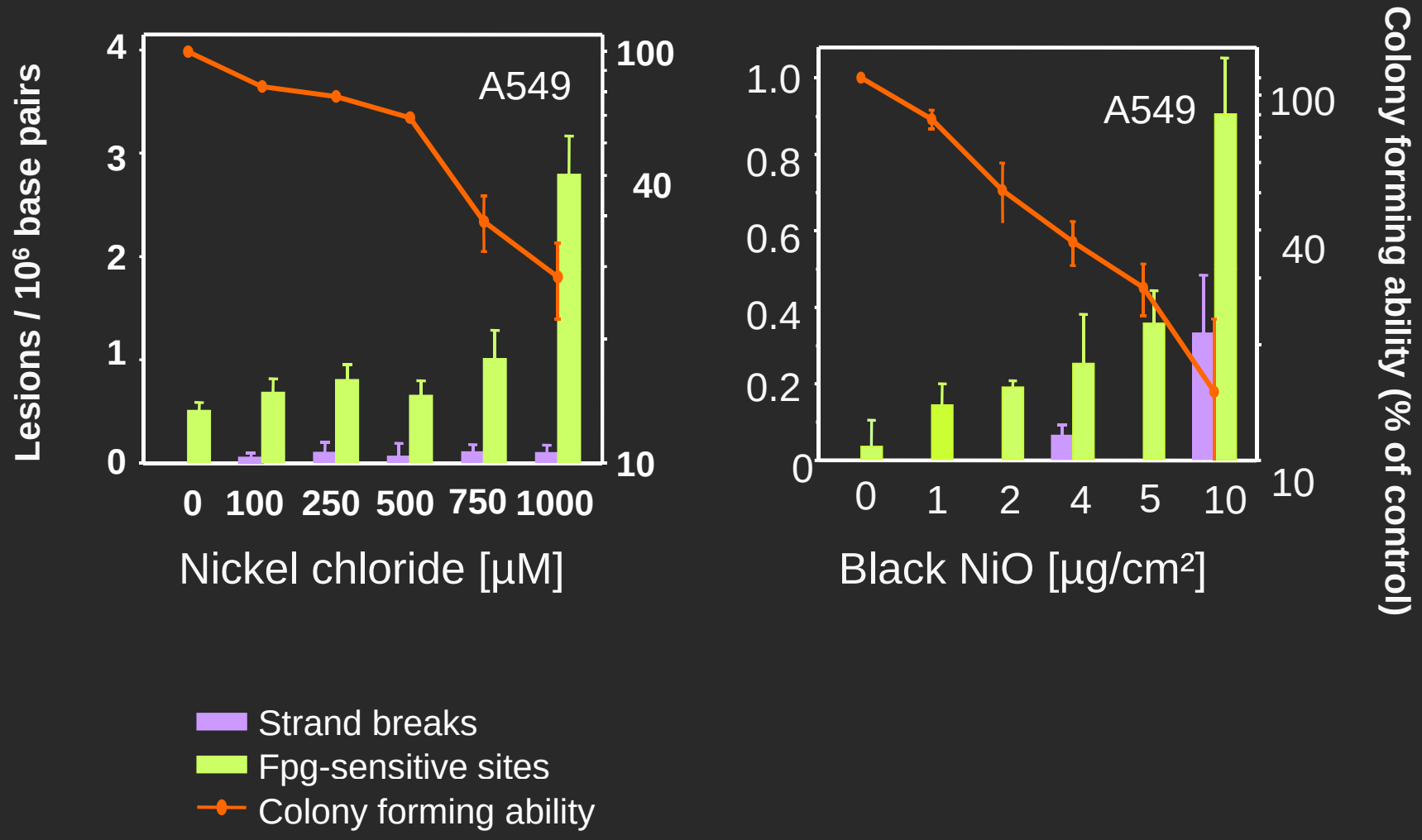


Cadmium:

→ **1000-fold higher affinity of XPAzf towards** Cd(II) as compared to Zn(II)

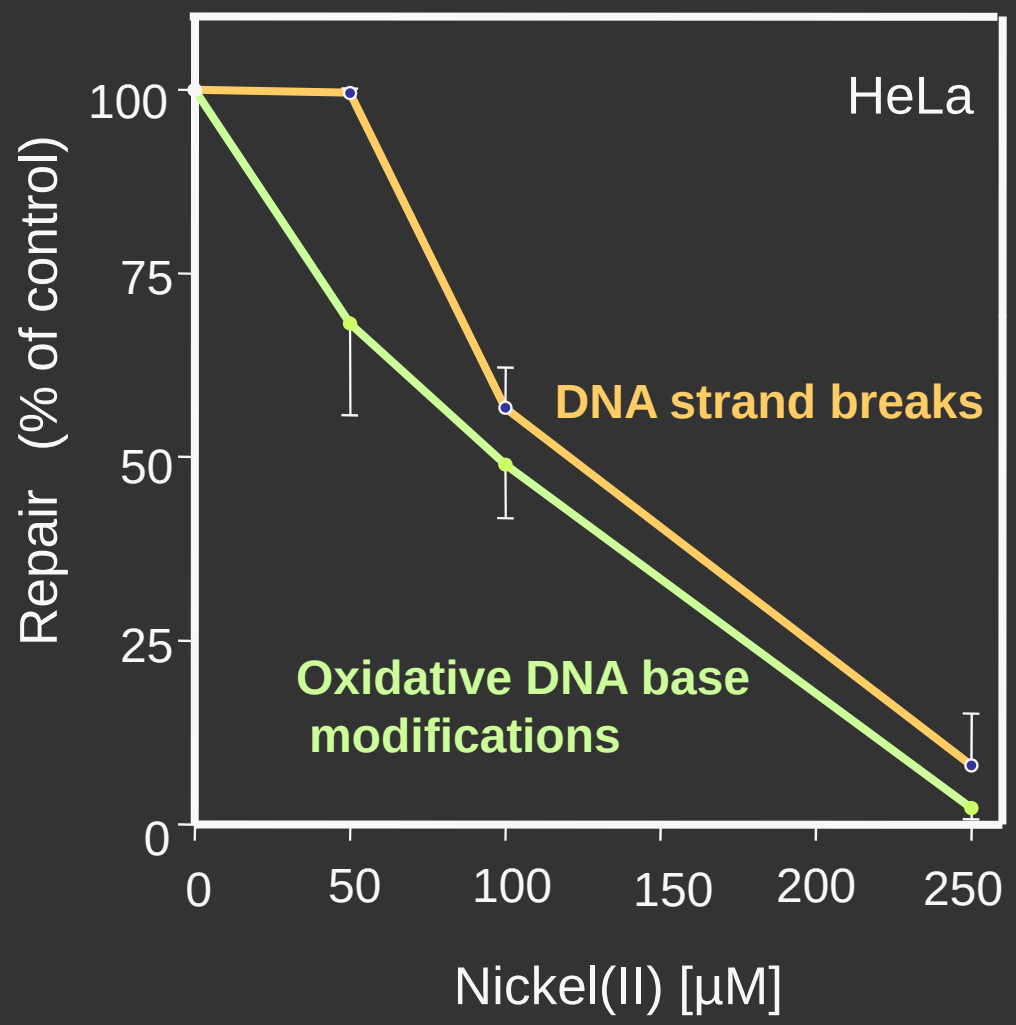
→ **Structural alterations** of the zinc finger domain (Kopera et al., Chem Res Tox 2004)

Induction of oxidative DNA damage by nickel compounds



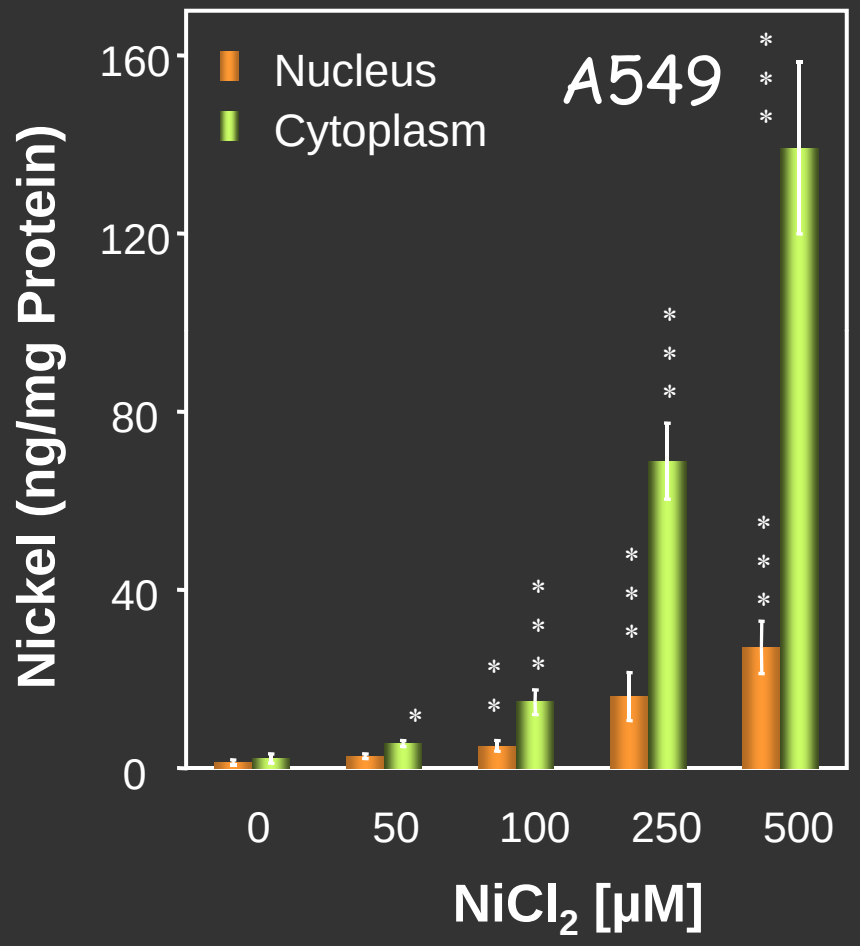
Schwerdtle, T. and **Hartwig, A.** (2006) *Materials Science and Engineering Technology*, **37**, 521-525.

Effect of Ni(II) on the repair of oxidative DNA damage induced by visible light

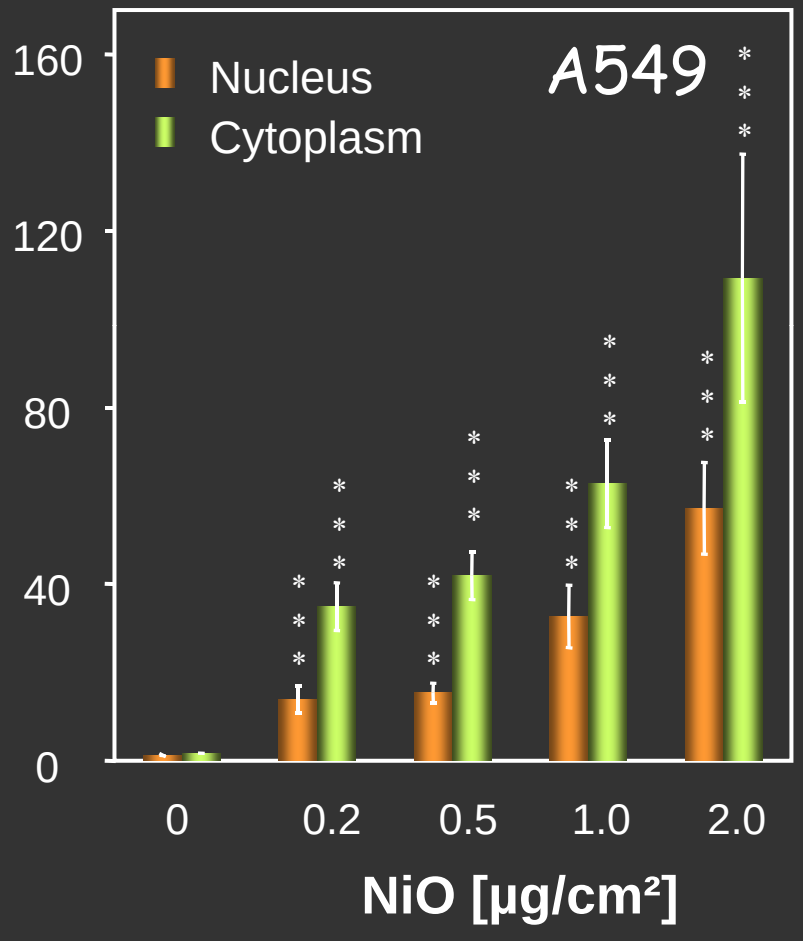


Dally, H. and **A. Hartwig** (1997) *Carcinogenesis,* **18**, 1021 - 1026.

Intracellular distribution of Ni: Comparison of water soluble and particulate compounds



20 h



24 h

Schwerdtle, T. and **Hartwig, A.** (2006) *Materials Science and Engineering Technology*, **37**, 521-525.

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The Nickel Ion Bioavailability Model for Respiratory Tumor Induction
by Nickel-Containing Substances

Presentation V
Dr. Joseph Landolph, Jr.

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Carcinogenicity of Nickel Compounds and Molecular Biology of Nickel-Compound Induced Morphological Transformation

Joseph R. Landolph, Jr., Ph. D.

Depts. of Molecular Microbiology/Immunology, Pathology, and Mol. Pharmacology and Pharmaceutical Sciences

USC/Norris Comprehensive Cancer Center

Keck School of Medicine/School of Pharmacy

University of Southern California

Los Angeles, California 91001

Telephone: (323)-224-7781

E-mail: landolph@usc.edu

Specific Insoluble Nickel Compounds Are Carcinogens

1. Epidemiological studies: Inhalation of dusts /aerosols of mixtures of insoluble + soluble Ni compounds by Ni refinery workers correlates with increased incidence of lung/nasal sinus cancers.

2. Inhalation of specific insoluble Ni compounds is carcinogenic in animals.

3. Ni compounds are found in emissions of oil/coal-fired power plants and in $PM_{2.5}$/PM_{10} air pollution particles and contribute to air pollution-induced lung cancer (along with Cr, Mn, etc.).

Questions Posed:

1. **Can specific insoluble nickel compounds induce morphological and neoplastic transformation of C3H/10T1/2 cells?**

2. **Can nickel compounds mutate 10T1/2 cells to ouabain resistance? If so, does mutation correlate with transformation in this cell system?**

Chemically Induced Multi-Step Neoplastic Transformation

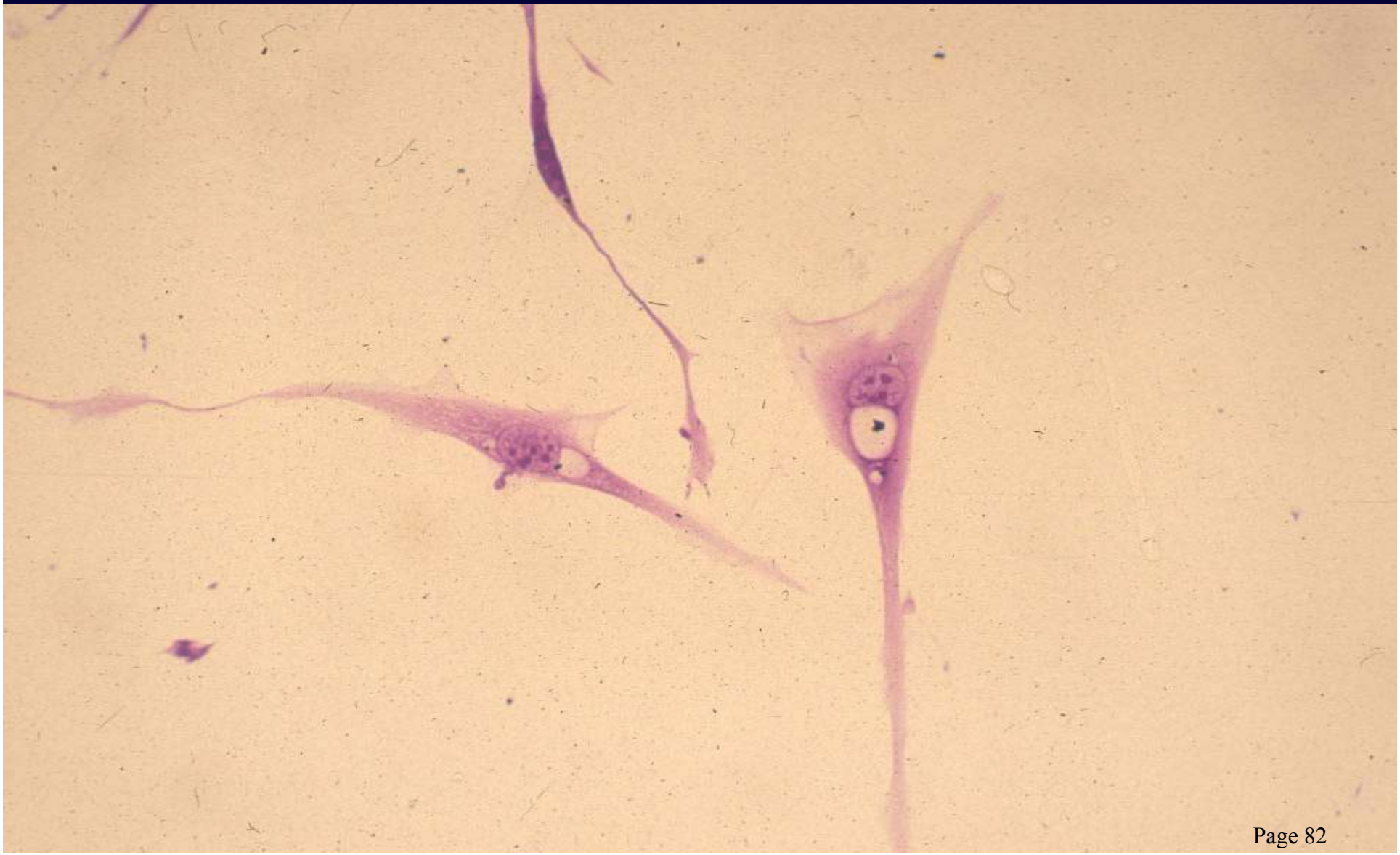
NiS

C3H/10T1/2 cells → Foci →

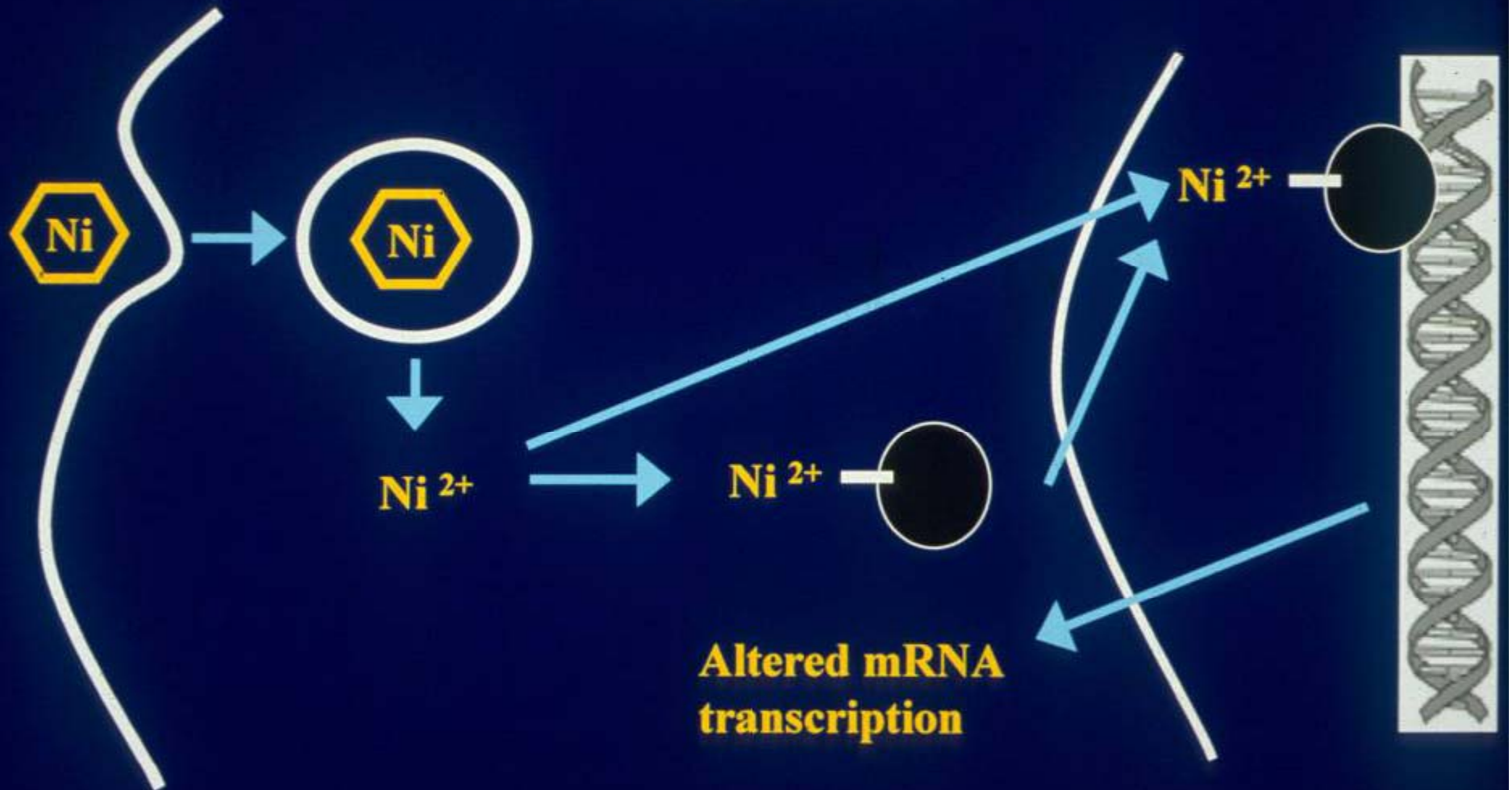
Anchorage Independence → Neo-

plastic Transformation

Phagocytosis of Ni_3S_2 Particles

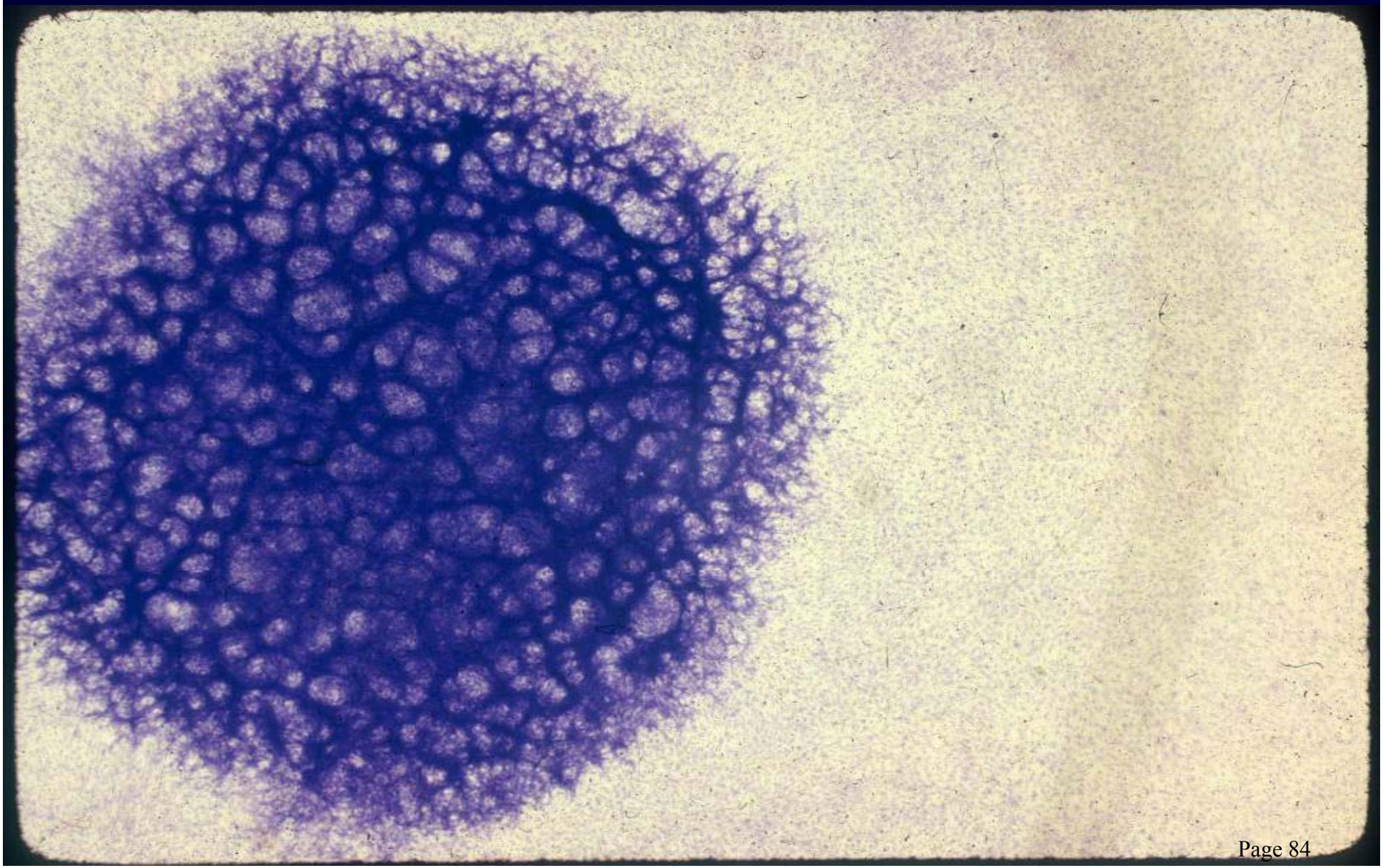


Cellular Uptake of Insoluble Ni Compounds



Landolph, J., Verma, A., Ramnath, J., and Clemens, F. Env. Health Persp., 110(S5), 845-850, 2002.

Focus of Morphologically Transformed Cells



Correlation of Ni-Induced Morphological Transformation with Ni Carcinogenicity

Compound	Morphological Transformation	Carcinogenicity
Ni_3S_2	+	+
NiO (green)	+	+
NiO (black)	+	+
NiS (crystall.)	+	+
Ni^0	+/-	+/-
$NiSO_4$	+(w)	-

Genotoxicity Profile of Nickel Compounds

Com-pound	Mutat. Oua^r	Mutat. 6TG^r	Micro-Nuclei	Chrom Aberr.	Morph. Trans.
Ni_3S_2	-	-	+	+	+
NiO(G)	-		+	+	+
NiO(B)			+	+	+
NiS(cr)					+
$NiSO_4$			+(w)	+(w)	+(w)
Ni^0				+	+/-

Conclusions

- 1. Insoluble Ni compounds are taken up into 10T½ cells by phagocytosis, and cause cytotoxicity, chromosome breakage, micronucleus formation, gene amplification, and morphological and neoplastic cell transformation.

Conclusions

2. Nickel-induced morphological cell transformation correlates well with uptake of particles of insoluble Ni compounds and Elemental Ni (Correlation Coefficient = 0.97).

3. Ni^{+2}-induced morphological transformation correlates with carcinogenicity of insoluble Ni compounds (Ni_3S_2 /NiO are carcinogenic in animals, $NiSO_4$ is not; Ni^0 particles are negative/weak).

Conclusions

4. Induction of Morphological Transformation correlates weakly with induction of Cytotoxicity (Correlation Coefficient, 0.55), not with Micronucleus Formation (Correlation Coefficient, 0.12) not with Mutation (to Ouabain Resistance; none induced so far).

5. Assay of Ni compounds to be phagocytosed into and cause morphological transformation in 10T1/2 cells can be used to detect carcinogenic Ni compounds and prioritize Ni compounds for carcinogenicity testing in animals.

Molecular Mechanisms of Morphological/ Neoplastic Transformation Induced by Insoluble Ni Compounds

Combination of:

1) Phagocytic uptake of Ni compounds,
2) Release of intracellular Ni^{+2} ions,
3) Ni^{+2} ion-generated hydroxyl radicals, radical-induced DNA damage, mutations, chromosomal aberrations, and
4) Ni^{+2} ion-induced hyper-methylation of promoters of tumor suppressor genes.

Studies of Two Samples From a Nickel Refinery in Wales

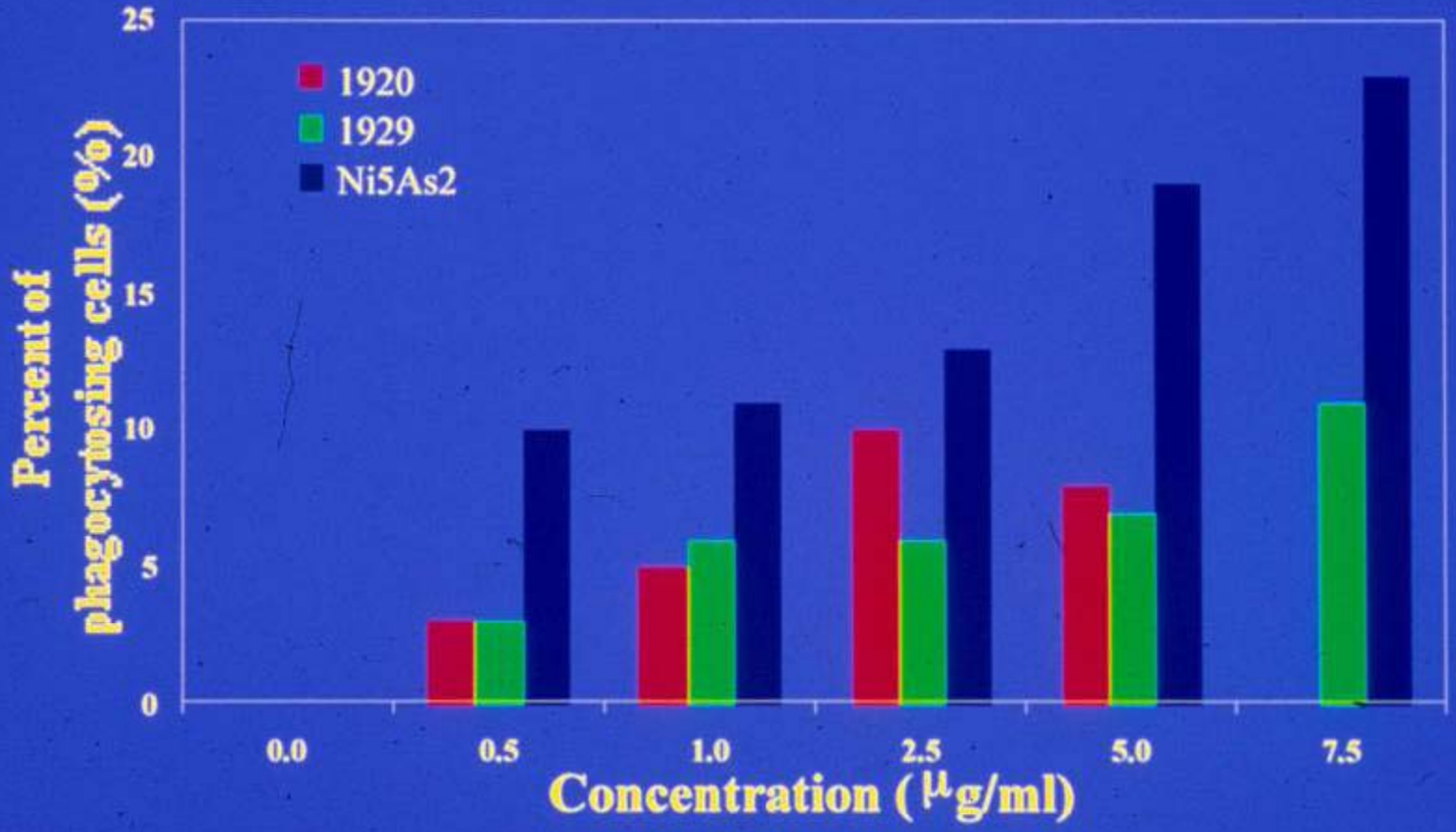
1. Workers in INCO nickel refinery in Clydach, Wales, U. K., developed nasal and lung cancers in l920's.
2. Sample from this refinery was taken and archived in l920 (CLYD3).
3. Refining process changed in l923. As-contaminated sulfuric acid was replaced.
4. Cancer incidence decreased significantly.
5. Sample from this refinery was taken and archived in l929 (CLDY23).

Comparison of Refinery Dust Samples from an INCO Nickel Refinery In Clydach, Wales

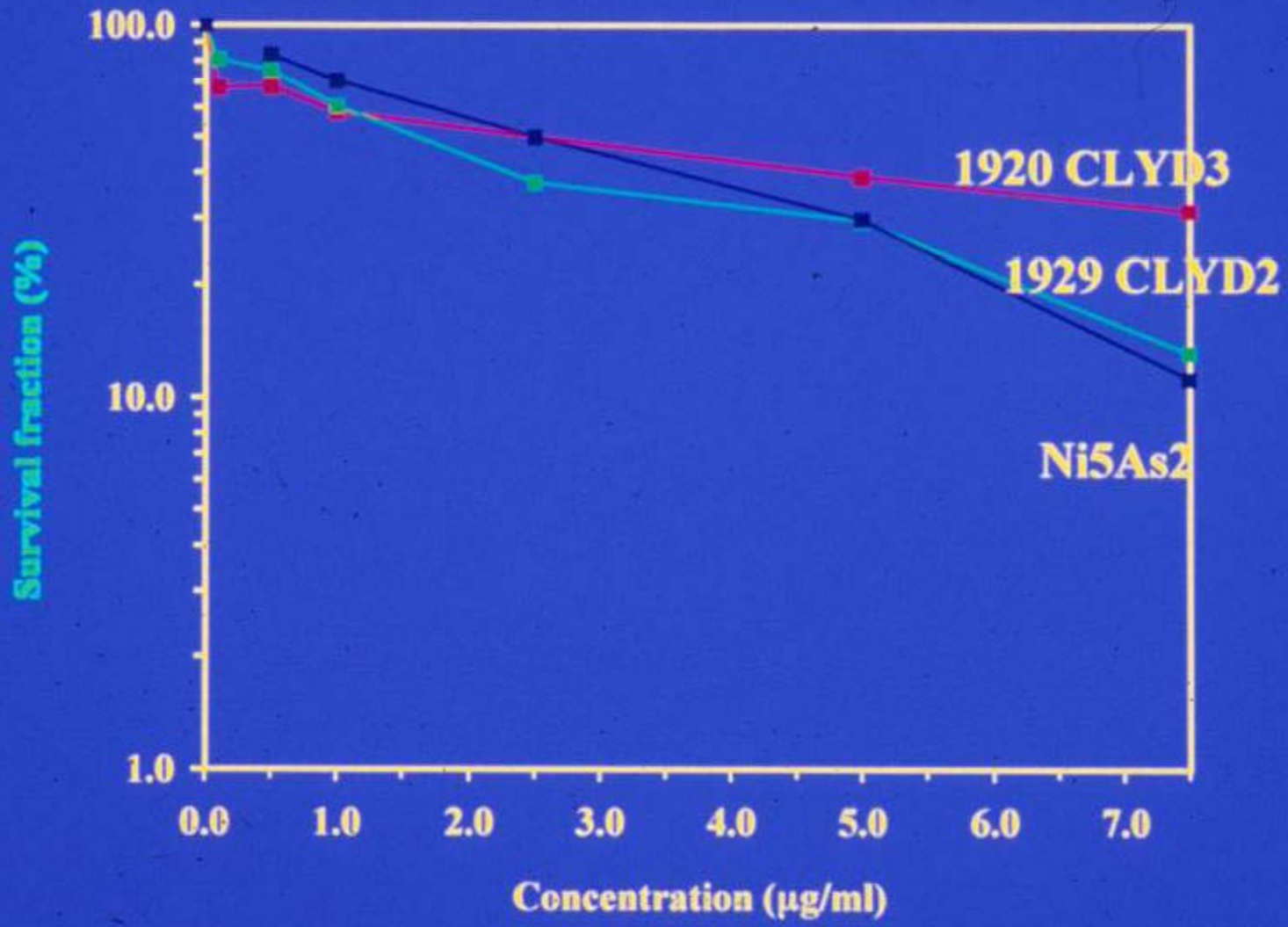
	CLYD3 (l920)	CLYD23(l929)
Percent nickel	**37.4%**	**26.6%**
Major components	**NiO**	**NiO**
	$Cu_{0.2}Ni_{0.8}O$	**$Cu_{0.2}Ni_{0.8}O$**
Minor components	**Fe_2O_3/Fe_3O_4**	**CuO**
	NiO•CuO	**NiO•CuO**
	As (~10%)	**As (~1%)**
	Ni_5As_2 (25%)	**Ni_5As_2 (2.5%)**

Both dust samples contain green NiO as major component. Main difference is a nickel arsenide (orcelite) in 1920 sample (Draper *et al.*, 1994b).

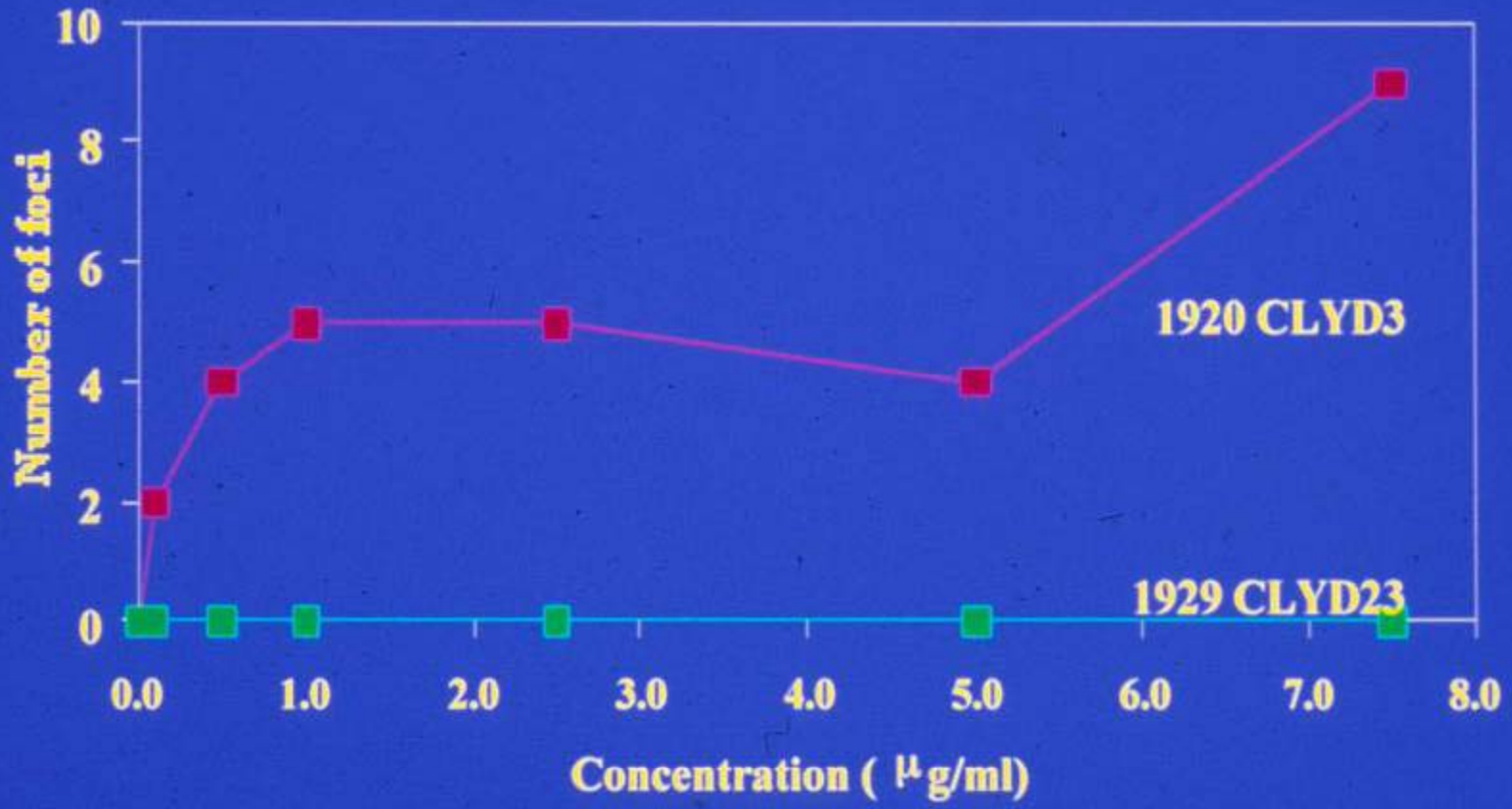
Phagocytosis Plot



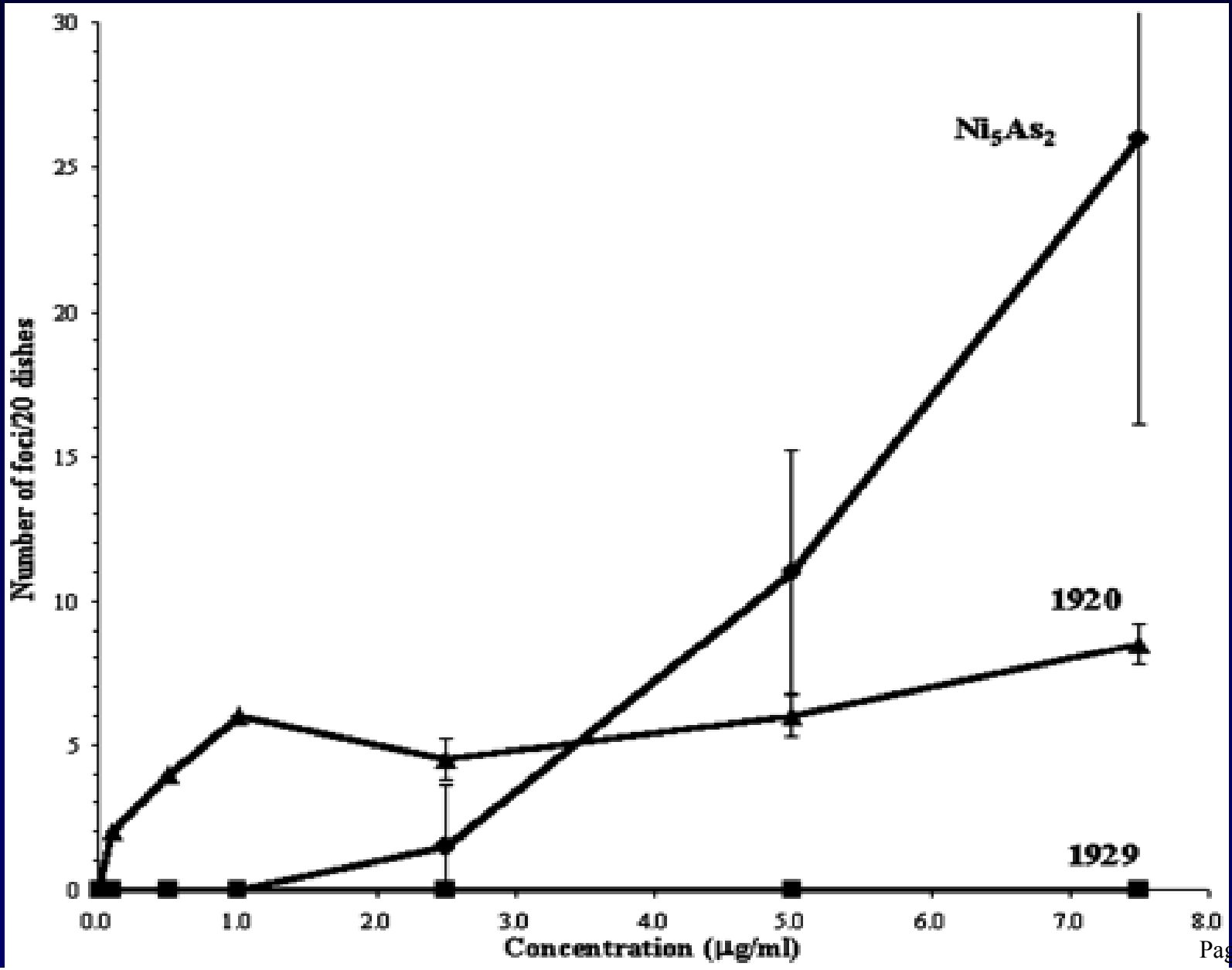
Cytotoxicity Plot



Transformation Plot



Morphological Cell Transformation



Genotoxicity of Clydach Samples

Com-pound	LC_{50}	Phagocyto-sing Cells at 5 ug/ml	Transform-ed Foci (Slope of Curve)	Chromos-ome Aberra-tions at 1 ug/ml
CLYD3 (I920)	3.2 ± 0.7	8.0	4.5, 0.38*	0.0 %
CLDY23 (I929)	1.9 ± 0.2	7.0	0.0	1.5 %
Ni_5As_2	2.5 ± 0.0	18.5	0.18*	1.5 %
Green (HT) NiO	3.1 ± 1.2	48.0	4.9	3.0 %

Conclusions

1. Epidemiology data shows increased incidences in human lung/nasal sinus cancers in this plant from 1916-1923. Refining process changed in l923 (reduced arsenic). No increased cancer incidence after l929.
2. l920 sample induced morphological transformation; l929 sample did not; this correlates with the epidemiological data.
3. Orcelite (Ni_5As_2) plus green NiO in l920 refinery sample, are likely responsible for induction of human lung and nasal sinus cancers in workers from Clydach refinery.

- Clemens, F., and Landolph, J. R. Genotoxicity of Samples of Nickel Refinery Dust. Toxicological Sciences, 73: 114-123, 2003.

The Nickel Ion Bioavailability Model for Respiratory Tumor Induction
by Nickel-Containing Substances

Presentation VI
Dr. Adrianna Oller
Oller, Schwerdle and Hartwig, 2006

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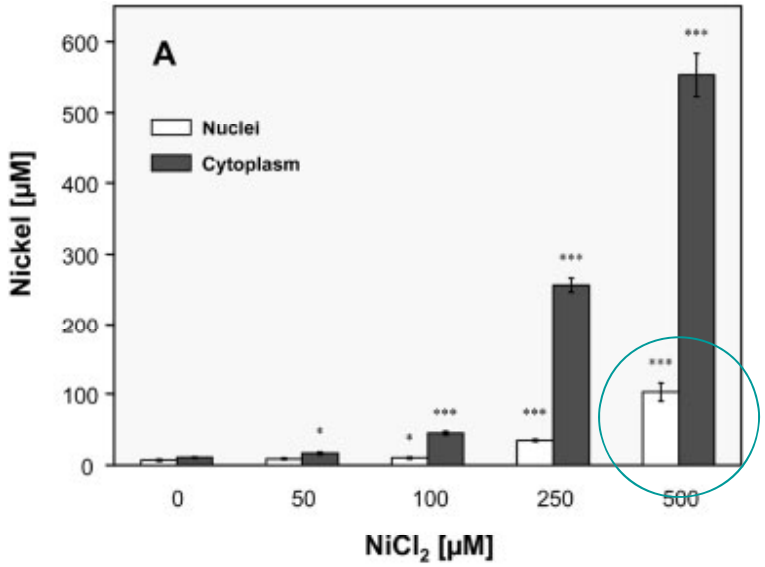
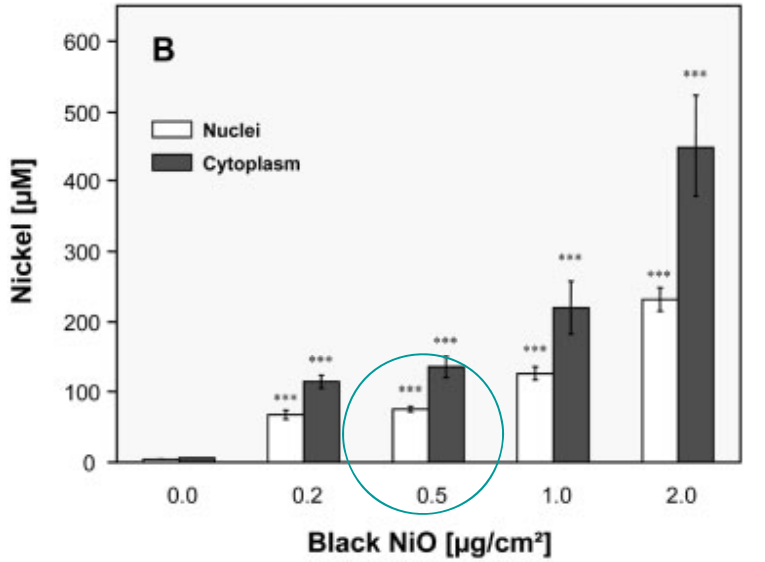
Schwerdle and Hartwig, 2006
"Bioavailability and genotoxicity of soluble and particulate nickel compounds in cultured human lung cells" Figure 2





Compare

What levels of Soluble Ni Compound are needed to achieve same intranuclear Ni levels as can be achieved with Ni Oxide?

	Concentration	Available Ni/ Petri dish	Concentration of Ni in nucleus
Ni chloride	500 µM	294 µg Ni	~ 100 µMolar
Nickel oxide	0.5 µg/cm^2	31 µg Ni	~ 75 µMolar

In vitro, and without clearance, ~7-times higher nickel from nickel chloride than from nickel oxide is required to achieve the same concentration of nickel in the nucleus of human lung cells.

If in the case of insoluble nickel oxide less than 31 ug Ni would be available to the cells for uptake because of deposition of particles on the bottom of the plate, then the difference between chloride and oxide would be even higher!

What levels of Ni compounds can be achieved in vivo?

	Exposure levels in rats in carcinogenicity study (mg Ni/m^3)	Tumors	Ni lung burden in rats after 15 month exposure (µg Ni/g control lung)
Ni sulphate	0.11	No	< 2
Nickel oxide	1.0	Yes	1,100

In vivo, the nickel lung burden from exposure to nickel sulfate at the MTD was more than 550-fold lower than the lung burden for nickel oxide at the lowest exposure level at which tumors were found for nickel oxide in rats.

Taking in vitro and in vivo data into account:

- *7 x 550 = 3850*
- Taken together the data suggest that in order to achieve in lung cells of rats the same nuclear nickel levels at which nickel oxide (1.0 mg Ni/m^3) induced tumors, rats would need to be exposed to 3,850-fold higher soluble nickel levels than the MTD of 0.1 mg Ni/m^3.
- In other words, they would need 550-fold higher exposures than the MTD to get same lung burdens as nickel oxide and then another 7-times higher exposure to get the same nuclear Ni levels as nickel oxide.
- 3,850-fold higher soluble nickel levels than 0.11 mg Ni/m^3 (equivalent to the most conservative HEC of 0.4 mg Ni/m^3) are unlikely be achieved in humans !

Appendix C: Additional Materials

Workshop on Nickel Ion Bioavailability

February 15-16, 2010

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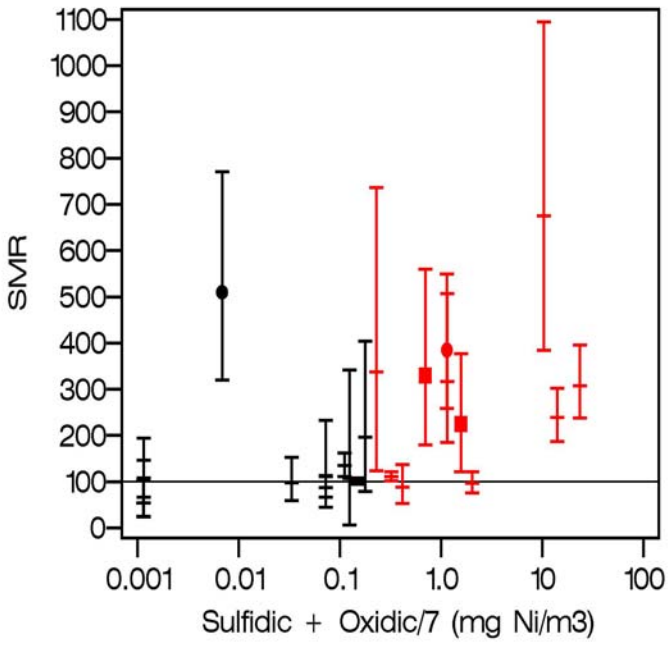
Suggested Graphs for Epidemiological Data – S.K. Seilkop

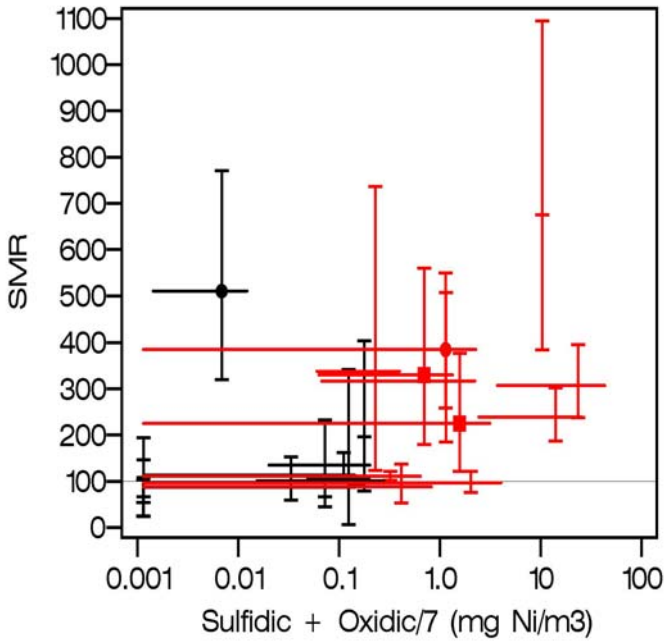
These are the graphs of exposure and lung cancer data from Tables 3 and 4 of Julie Goodman's paper that I promised at the workshop:

- The graphs on the left side of the page show estimated standardized mortality ratios for the available epidemiological data at the mid-points of the estimated exposure ranges for the different workplaces that are represented. The graphs on the right side of the page show the same data with the addition of horizontal lines representing the estimated exposure ranges.
- The exposure data are shown on a logarithmic scale to allow better differentiation at the low end of the exposure range; as messy as the graphs are, they were much worse using the natural scale. Please note that exposure concentrations reported as 0 mg Ni/m3 are shown as 0.001 mg Ni/m3.
- The lines in red show cohorts which would be predicted to have excess lung cancer risk, based on exposures at or above human-equivalent LOAELs estimated from NTP animal bioassay data for nickel subsulfide and nickel oxide.
- The graphs on the first page show lung cancer response relative to the sum of estimated sulfidic nickel concentrations plus estimated oxidic nickel concentrations divided by 7 (to reflect the differential in tumorigenic potency of nickel oxide relative to nickel subsulfide). The graphs on the second and third pages show lung cancer response relative to the sum of soluble nickel equivalents (in alveolar and interstitial fluid) across the different nickel substances to which workers were exposed.
- The data shown on the graphs represent different work areas, apart from roasting/smelting and electrolysis at Kristiansand, which had lung cancer risk and exposure estimates from two different studies (Grimsrud et al. and Andersen et al.).

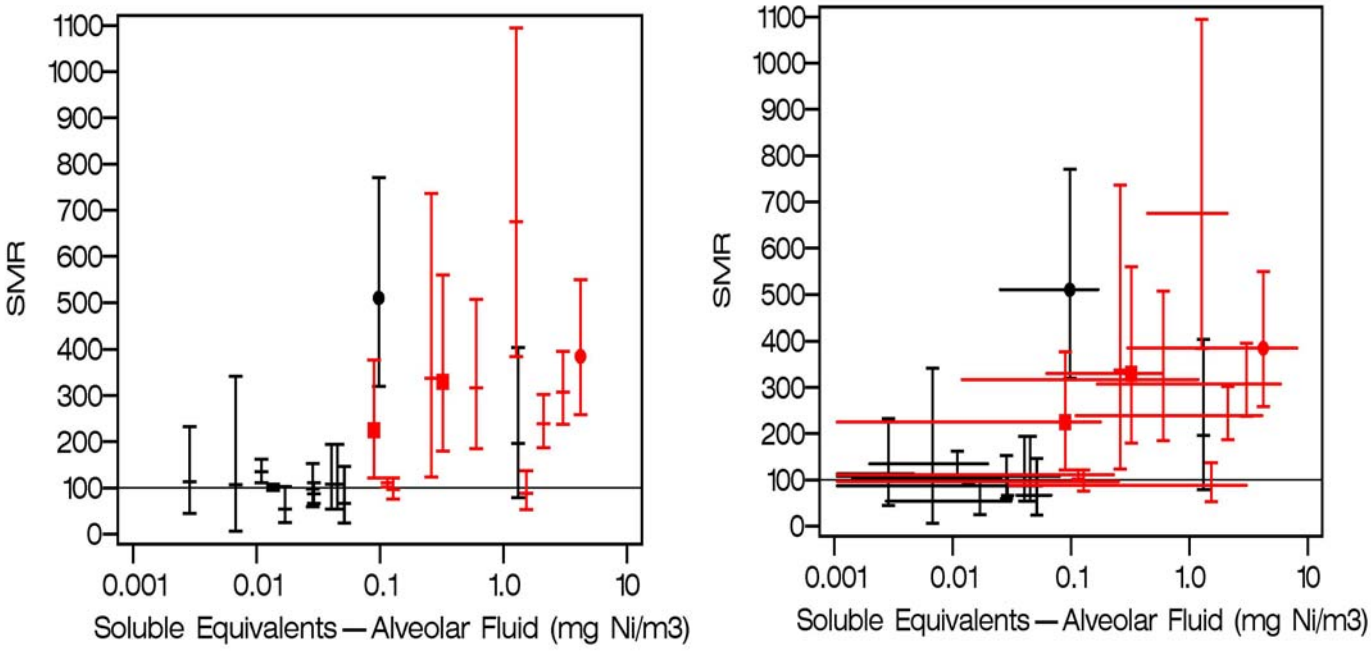
My interpretation of these data is that:

- Both sets of graphs (bioavailability based - sulfidic/oxidic; nickel ion based - soluble nickel equivalents) show evidence of exposure-response gradients, but with a substantial degree of inconsistency.
- Much of the inconsistency in the graphs is undoubtedly attributable to uncertainty/inaccuracy in estimated exposure concentrations. For example, the two lowest (non-significant) SMRs shown in red have exposure concentrations for both sulfidic/oxidic and soluble nickel equivalents that are between 0.001 mg Ni/m3 and the reported "best" mid-range estimate. It would therefore not be unreasonable to assume that the actual average exposure was considerably less than the estimated value (and also below the animal estimated LOAEL for sulfidic/oxidic nickel). Some error in exposure estimation is clearly structural - for example, in the sulfidic/oxidic graph, the rightmost of the two non-significant SMR's in red is for Huntington Alloys before 1947. The location of this SMR reflects sulfidic exposure in the calcining operation, while the SMR itself reflects lung cancer in the entire cohort (of which calcining workers were a relatively minor subset). The problem of uncertainty in exposure estimation is further exemplified by the electrolysis workers at Kristiansand studied by Grimsrud et al. and Andersen et al. Across these two studies, estimated insoluble nickel exposures in electrolysis workers differed by more than two orders of magnitude, and soluble exposures differed by a factor of approximately 50.
- Considering the uncertainties in the data, it could be argued that either the bioavailability model or the nickel ion theory is consistent with the observed epidemiological evidence. However, the plausibility of the nickel ion theory is somewhat more doubtful. This is based on: 1) negative animal carcinogenicity data for soluble nickel, and 2) in every instance of substantially increased human lung cancer risk associated with high soluble nickel exposures (apart from the Grimsrud et al. estimated of risk in electrolysis workers), concomitant estimated exposure to sulfidic and/or oxidic nickel was sufficiently high to result in increased risk, based on predictions from animal bioassays.
- Overall, however, the uncertainties in the epidemiological data (particularly with respect to exposure estimation) preclude a definitive determination of the veracity of the alternative models/hypotheses.





LEGEND:
Vertical bars depict 95% confidence intervals for SMRs
Horizontal bars show estimated ranges of exposures
— Exposure < HEC LOAEL for tumors in rats induced by nickel subsulfide or nickel oxide
— Exposure ≥ HEC LOAEL for tumors in rats induced by nickel subsulfide or nickel oxide
●, ● Estimates for electrolysis workers at Kristiansand from two different studies (● Grimsrud et al., ● Andersen et al.)
■ Estimates for roasting and smelting workers at Kristiansand from two different studies



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Vertical bars depict 95% confidence intervals for SMRs
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— Exposure < HEC LOAEL for tumors in rats induced by nickel subsulfide or nickel oxide
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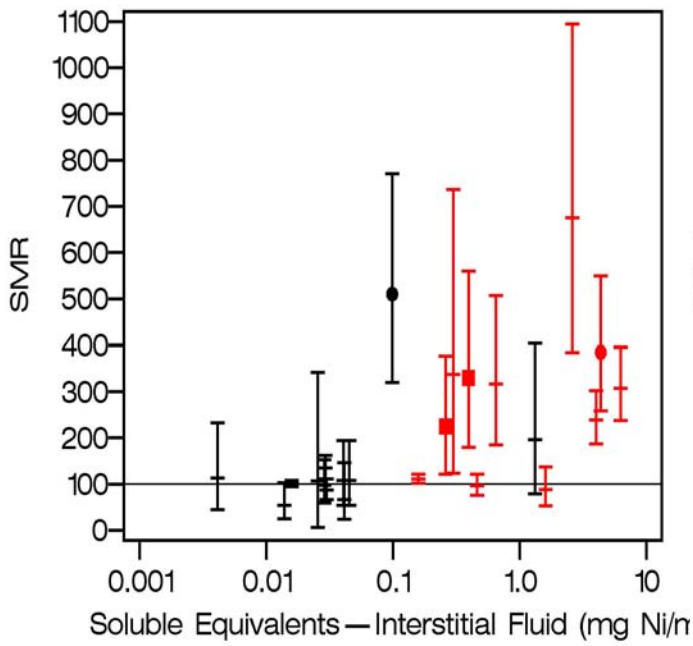

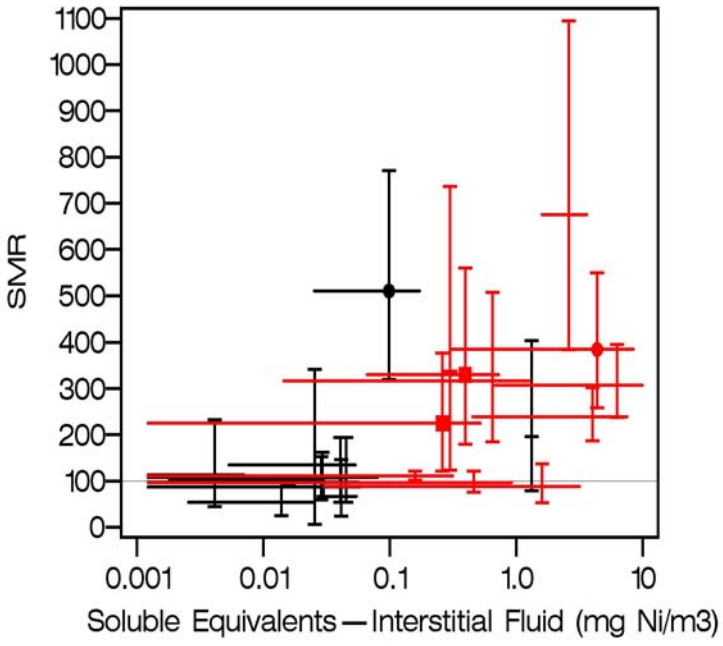

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