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Before the U.S. Senate Subcommittee on Environment and Public Works: Full Committee Hearing
Entitled **“Oversight Hearing on the Environmental Protection Agency’s Implementation of the
Safe Drinking Water Act’s Unregulated Drinking Water Contaminants Program”**.

Tuesday, July 12, 2011; EPW Hearing Room – 406 Dirkson

Chairman Boxer, Ranking Member Inhofe, and members of the committee, thank you for the opportunity to appear before you today to testify on EPA’s work on the Safe Drinking Water Act’s Unregulated Drinking Water Contaminants Program, specifically with respect to hexavalent chromium.

My name is Steven Patierno and I am the Executive Director of The George Washington University Cancer Institute (GWCI) and the Vivian Gil Distinguished Professor of Oncology at the George Washington University. I am also a Professor of Pharmacology and Physiology, and Genetics at The George Washington University School of Medicine and Health Sciences; a Professor of Environmental and Occupational Health at The George Washington University School of Public Health and Health Services; and the Founding Director of the Molecular and Cellular Oncology Program. I am actively engaged in several areas of cancer research and intervention including drug discovery, cancer health disparities, patient navigation and cancer survivorship, but what is most relevant to today’s discussion is that I have been conducting research on hexavalent chromium for 31 years and my basic science laboratory has been funded by the National Institute of Environmental Health Science and/or the National Cancer Institute, continuously for 23 years, to study cellular and molecular mechanisms of hexavalent chromium toxicology and carcinogenesis. I have published more than 120 peer-reviewed scientific papers and have served on numerous review panels for hexavalent chromium risk assessment including both the previous and the current EPA Toxicological Review of Hexavalent Chromium in support of the Summary Information on the Integrated Risk Information System (IRIS). I would also like to disclose that although I have never worked or consulted directly with or for any company associated with the chromium production or use industries, over the course of 30 years working in chromium toxicology I have, on seven occasions, served as an expert for the defense in chromium litigation.

A recent press release issued by the Environmental Working Group, entitled “Chromium-6 in U.S. Tap Water” reported that very low levels of hexavalent chromium were found in drinking water from 31 U.S. cities. The average level reported was 0.18 ppb with a range of 0.03 to 13 ppb. Interestingly, most of the cities with the highest levels of ambient Cr(VI) have little or no proximity to

any chromium-related industry, indicating that these low levels constitute a natural background. Given that chromium is a natural component of the earth's crust, these results were neither new nor unexpected. The EWG report and the associated media coverage, which was purposeful in referring to Cr(VI) as the "Carcinogenic Erin Brockovich Chemical", has caused unnecessary fear and alarm, as these levels constitute no health risk to humans.

Before analyzing this low level exposure scenario it should be stated that there is a vast literature on occupational and industrial exposures to high doses of chromate compounds for long periods of time, as encountered in the chromate production and plating industries in the early to mid-1900s. There is also a very large literature on the effects of chromate compounds in animals and in defined systems such as cell culture. Valid conclusions are typically drawn when complementary data from different types of studies indicate that an observed effect is reproducible, dose-dependent, free from confounding variables and statistically significant. Concepts which are fundamental to the principles and practice of pharmacology and toxicology, including dose, duration of exposure, route of exposure, metabolism, toxicokinetics and detoxification, must also be factored into an accurate analysis.

Taken together, the consistent message is that only long-term, high dose exposures to moderately or highly insoluble particulate forms of either chromate dusts or concentrated chromic acid mists, (or in the words of the International Agency for Research on Cancer [IACR], "as encountered in the chromate production, pigment and plating industry") have been associated with human cancer and even then, only for cancers of the respiratory tract. These same studies, which consistently detected a positive correlation with respiratory cancer, also showed there is no consistent association with any increased risk for any other cancers. This is attested to by every major government or international agency-related review ever written including the International Agency for Research on Cancer (IARC), the previous US EPA Toxicological Review, and the Agency for Toxic Substances and Disease Registry (ATSDR).

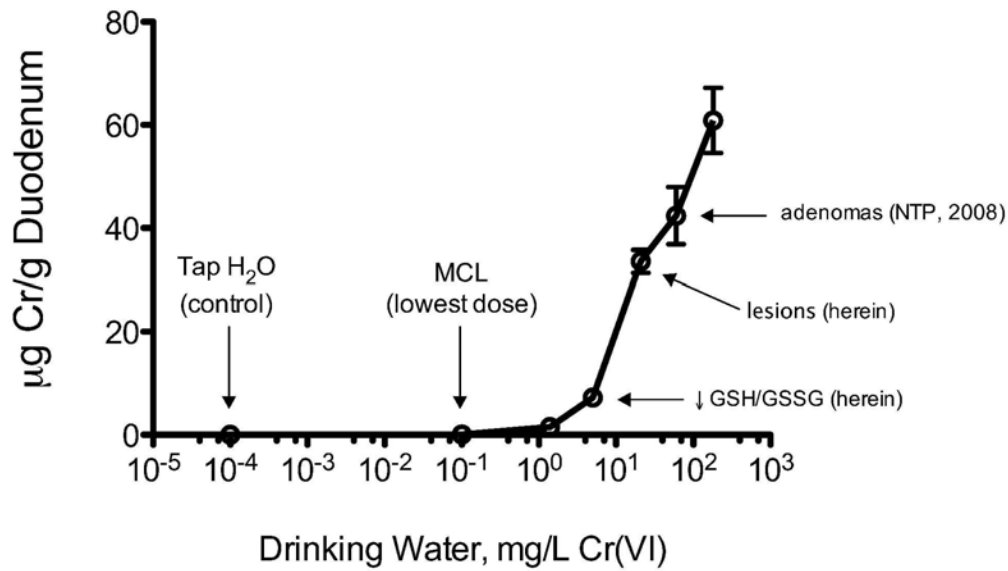
Furthermore, all three major areas of risk-related study, (epidemiology, animal and cell culture) provide clear evidence for a very high threshold levels for both toxicity and carcinogenesis. For example, in an analysis of occupational risk in a cohort of more than 2300 workers in the chromate production plant by Gibb et al, widely cited as a reference paper for risk assessment, no increased risk for lung cancer was observed in long-term occupationally exposed workers exposed to concentrated mixtures of chromic acid mist and/or dusts of chromate particulates at a mean exposure level of 450 ng/m³-yr (Odds ratio of .96 factored over a 45 year work history). These exposures were high enough to cause severe nasal tissue damage including perforation of the nasal septa. At more than a 9-fold increase in mean exposure (4,200 ng/m³-yr), the Odds ratio for

risk was increased to 1.42 but the increase was still not statistically significant. Statistical significance must be achieved in order to validate that a given observation is real and not the result of chance. Only when exposure was 7-fold higher again (30,000 ng/m³-yr), did the odds ratio for risk achieve statistical significance at 1.57. It should also be noted that 116 of 122 workers who developed lung cancer after long-term, high-dose exposure, were also smokers.

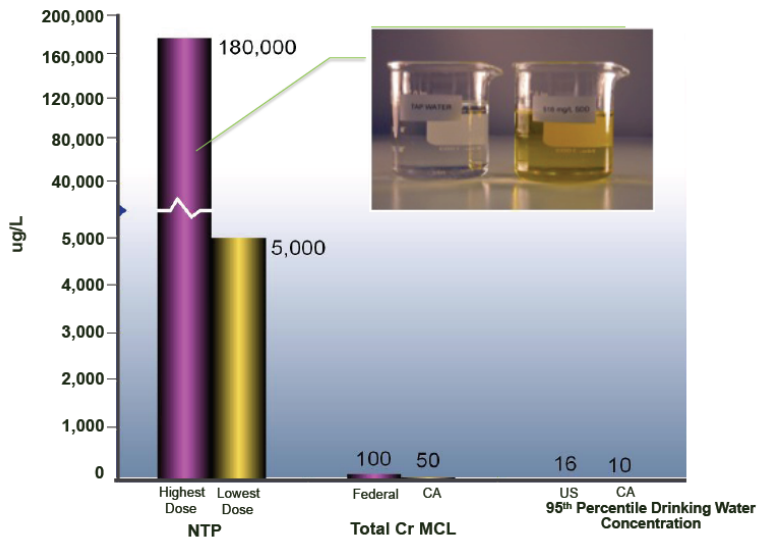
Likewise, a published Meta-Analysis investigated Gastrointestinal Tract cancers (oral, esophageal, stomach, small intestine, colon and rectal) in all epidemiology studies of Cr(VI) exposed workers published after 1950 (Gatto et al. 2010), including 32 studies from various industries wherein airborne concentrations were extremely high and resulted in oral exposure as evidenced by yellow stained teeth, tongues and GI distress showed no significant increased risk for malignancy at any GI site.

An additional often-overlooked fact is that Dr. John Morgan, an excellent epidemiologist working for the State of California Cancer Registry, has been tracking cancer incidence in the town of Hinckley CA (the “Erin Brockovich” town) for the past 15 years. He recently reported that not only is there no excess of total cancer or any specific type of cancer in Hinckley, there are actually fewer cancers than expected.

Further evidence for a very high threshold exposure level for Cr(VI) carcinogenesis is found in rodent carcinogenesis bioassays conducted by the National Toxicology Program (Stout et al, 2009) and in a multi-center analysis of Cr(VI) Mechanism of Action by ToxStrategies (first paper just published by Thompson et al, 2011). In the NTP study mice and rats were exposed to extraordinarily high concentrations of Cr(VI) (0, 14, 57, 172, or 516 ppm of sodium dichromate dihydrate) in the drinking water continuously for two years. In the NTP study, tumors were only observed in the small intestine of mice at the highest two dose groups, relative to concurrent controls, and at all the doses in the NTP study, the high concentration exposures resulted in long-term tissue damage in these tissues. Thompson et al. 2011 administered Cr(VI) in drinking water to mice at the same concentrations as NTP in the NTP study and at two lower concentrations, including the current Federal drinking water standard (the Maximum Contaminant Level or MCL). As shown below from Thompson et al. 2011, no toxicological effects were observed even at doses in the mouse small intestine that are nearly five orders of magnitude (100,000 times) higher than the average tap water concentration of Cr(VI) in that study, which was conducted in Birmingham, Alabama. The drinking water concentrations of Cr(VI) for the control animals ((not dosed with Cr(VI)) in the Thompson et al. 2011 study, and the NTP study which was conducted by the same laboratory, are consistent with the levels reported across the US in the EWP report.



The following figure shows the NTP study doses relative to the current MCL and the 95th percentile drinking water concentration in the US and in California for which extensive drinking water monitoring data exists. Note the break in scale for the highest dose.



It is important to understand that despite Hollywood depictions, Cr(VI) is not “potent” as either a toxin or a carcinogen and in fact if anything should be recognized as a very weak potential carcinogen. Enormous quantities of chromium(VI) are needed to evoke any kind of toxicity (demonstrated and documented by cases of accidental or suicidal poisonings) in humans or animals, and as mentioned above, respiratory carcinogenicity in humans is only associated with high-dose, long term occupational exposure to chromate dusts or concentrated chromic acid mists.

As stated by Paracelsus in the 16th century (who is widely recognized as the father of pharmacology and toxicology), “all substances are poisons and there is none which is not a poison, the right dose differentiates a poison from a remedy). For example, over-the-counter drugs from a local pharmacy (like acetaminophen) have either no effect or a therapeutic effect at low doses, but at high doses even such widely available drugs can be toxic or even lethal. In the same way humans have many-tiered, innate mechanism of protection against chemical toxicities of any sort, including Cr(VI).

To understand this, it is critical to understand that chromium compounds exist, for the most part, in two chemical forms called hexavalent and trivalent chromium. Trivalent chromium ((Cr(III)) is chemically very stable and is actually an essential element that is required for normal human physiology. We get large amounts of Cr(III) from our diet. After ingestion, very little of it is absorbed and the part that is absorbed into the body does not easily get into cells. Its essential activity as co-factor in insulin regulation seems to take place at the surface of cells but not inside. In contrast, hexavalent chromium is chemically similar to sulfate (another essential element) and it can slip inside of cells posing as sulfate. However, Cr(VI) is chemically unstable and easily converted to Cr(III) by a large number of natural component of our saliva, gastric juice, lung fluids, and blood components. Once Cr(VI) is converted to Cr(III) outside of our cells it behaves as an essential element and does not readily enter cells.

Recent studies using human gastric juice show that even large quantities of concentrated solutions of Cr(VI) are completely converted to Cr(III) in less than a minute. At low concentrations the conversion is almost instantaneous. This means that unless enormous concentrations of Cr(VI) employed, high enough to overwhelm the conversion capacity of our body fluids, little or no Cr(VI) will reach the surface of any cell. In addition, any tiny amount Cr(VI) that temporarily escapes instantaneous reduction would encounter the mucous lining of the respiratory tract and GI tract and not have easy access to the actual surface of any cell. If by chance a massive dose is administered, high enough to saturate the conversion capacity of lung or gastric fluids, some Cr(VI) may be absorbed into the bloodstream. There it will encounter an even greater capacity of our blood plasma to convert Cr(VI) to Cr(III) making it virtually impossible for Cr(VI) to arrive at other tissues as Cr(VI) except at massively toxic concentrations. This conversion capacity serves as a powerful barrier to Cr(VI) toxicity and its existence creates a very high threshold for Cr(VI) toxicity: it must be overwhelmed before toxicity can occur. The fact that all cells have a sulfate transport system that Cr(VI) can piggy-back on does mean that Cr(VI) can cause every type of cancer. One cannot ignore all the many-tiered protective barriers that prevent Cr(VI) from getting to surface of cells distal to the point of entry. Even at the massive doses administered in the NTP study, Cr(VI) did not cause cancer outside the GI tract.

Once Cr(VI) finally enters a cell it has to be converted to other forms of chromium inside the cell in order to become toxic. It also encounters additional barriers to becoming toxic because these other forms of chromium are quickly bound up by protective molecules inside of cells and rendered inactive. Only when an intracellular concentration of Cr(VI) is reached that overwhelms these protective barriers can it begin to interact with important macromolecules like DNA and protein. The excess Cr(VI) may damage these molecules through a process of chemical oxidation, usually leading to the destruction of the cell. Cr(III) formed inside of a cell by conversion from Cr(VI) is capable of binding to DNA and many studies, including from my own laboratory, have reported on what we thought was a mutagenic mechanism of action as result of DNA damage by Cr(III). However, for many years we have been concerned about the fact that very high doses and highly contrived experimental conditions, high enough to kill most of the exposed cells, were necessary to detect mutagenesis. Many of us, including eight of the nine current reviewers of the Draft Toxicological Review, have come to understand that what we thought was mutagenesis was in fact more likely a process of selection for chance survivors of the toxic treatment. The ninth member abstained, but only out of concern that the EPA's linear default model is a historical precedent that is not likely to be overruled. Nevertheless, it more likely that the carcinogenic MOA of Cr(VI) under high dose, long term exposure conditions is due to chronic tissue damage, inflammation and chronic regenerative cell proliferation. At doses lower than the threshold there simply is no MOA because there is no toxicity or carcinogenesis. These important concepts need to be considered by the EPA.

Before I conclude I need to address one other important issue. As one can quickly discern from the EWG report there are some who have taken to espousing the opinion that even vanishingly small, short term exposures to Cr(VI) are capable of causing a plethora of human diseases and virtual every type of cancer known to mankind. To support this premise reference is frequently made to two "opinion" articles in the literature, one in 1997 and one in 2006, published in the journal *Critical Reviews in Toxicology*. These articles were written and published at a time when the senior author was actively engaged as an expert witness for the plaintiffs in high-profile chromium lawsuits, but this involvement was not disclosed in the 1997 article. Production of the 2006 article was paid for by the plaintiffs law firm but this was only partially disclosed. These papers were cited by the Draft 2010 EPA Toxicological Review of Hexavalent Chromium, but premise of these papers is not accepted by the general scientific community and it was unanimously rejected by the current nine-member review panel of the Draft Toxicological Review because the methodology applied is severely flawed.

It is well established that when large scale epidemiological studies examining many

endpoints are conducted, random fluctuation due to the breadth of the study will result in number of false positives (random, usually small and nonstatistically significant increases in risk rates for specific cancer). Statistical significance is extremely critical in epidemiological studies because the number of comparisons in a typical epidemiological study make it inevitable that some increased SMRs or Odds Ratios would be arrived at by chance. One must look for consistency across multiple studies to determine whether it is real. In these articles whatever instances that could be found in any epidemiology study of chromium, of an elevated Standard Mortality Ratio (SMR) were picked and presented in tabular form with no consideration of the fact that most of these instances were small, non-statistically significant elevations that were ignored or discounted by the original authors because of confounding factors. The paper also failed to show that many of these random non-significant elevations in some cancers in one selected study were counter-balanced by either no elevation or decreased SMRs in other studies. This is incorrect scientific methodology but it illustrates the importance of critically evaluating epidemiological data from original journal articles and not relying on an opinion paper as the Draft EPA Toxicological Review apparently did.

Thank you for the opportunity to address you today.

Further Background on Chromium and Chromium Carcinogenesis

Examples of Additional Papers Incorrectly Cited to Suggest that Occupational Exposures to Cr(VI) can cause cancers other than Respiratory Cancer.

Another published paper that is sometimes cited (including in the 2010 Draft EPA Toxicological Review of Chromium) in an attempt to link environmental chromium exposure to Non-Hodgkin's lymphoma is Bick et al, *Int. J. Hematol.* 64:257-262, 1996). This paper should be retracted from the scientific literature. Two of the authors were lead lawyers for the plaintiffs in several high-profile chromium lawsuits, now immortalized by the Hollywood movie "Erin Brockovich". They listed their "academic" credentials as the Department of Hematology at the University of Tasmania in Australia. The other three authors were paid expert witnesses for the plaintiffs in the same case, which was active at the time. None of this was disclosed in the paper. The two cases of Non-Hodgkin's lymphoma discussed in this case report were plaintiffs in the active lawsuit and the information was supplied by the lawyers. Moreover, at best this report is merely a case-report (not even a case-control study), merely reporting that two people in Hinckley CA, at that time, had been diagnosed with Non-Hodgkin's lymphoma.

In contrast, for perspective it is important to look at historical occupational exposures that were associated with increased risk (summarized in the ATSDR Toxicological Profile for Chromium, by IARC, and by OSHA in the Federal Register). The history of the recognition of certain chromium compounds in lung and other respiratory cancers traces back to Scotland in the late nineteenth century and to Germany in the 1920's. In the work environment of the 1920's through 1950's, the levels of dust in factories were so high that it was said to be difficult to see across the factory floor (hundreds of micrograms to milligrams of chromium per cubic meter of air). Workers had no protective gear and they would leave work with chromate dust encrusted on their clothes and in their noses. Much of the dust would be inhaled and swallowed. Worker safety protocols and health monitoring were non-existent. The increases in lung cancer provoked the application of modern industrial hygiene practices in these industries, and by the 1960's most Western plants using chromium had instituted industrial hygiene practices that dramatically reduced exposure to airborne particulate chromates and virtually eliminated the adverse health outcomes associated with chromium exposure.

The consensus of scientific opinion, summarizing a very large number of epidemiological studies, animal studies and mechanistic studies, is that an increased risk of lung cancer can be associated with long-term, high dose exposure to either acid mists of soluble Cr(VI), or highly insoluble particulate dusts of calcium, lead or zinc chromate, as they were encountered in occupational settings such as mining or production industries. Even under such heavy exposure conditions, there was no association of exposure with increased risk for cancer of any other organ system other than lung cancer, and most of the chromium-exposed workers were also smokers. Among soluble chromates, only occupational inhalation exposure to concentrated mists of chromic acid in the chrome plating industry were associated with increased risk for respiratory cancer.

For example, in risk-associated production industries (dichromate and chromium trioxide) and pigment industries, men were exposed to concentrated dusts of the low solubility particles of lead chromate and zinc chromate. In some plants, exposure levels were found to range from 10,000 to 190,000 ng/m³ for an average of 18 years. In some plants, CrVI exposures averaged around 170,000 ng/m³, often for more than 20 years. Some risk was noted after a two year exposure in a plant with exposure levels above 400,000 ng/m³. In other plants, multi-year exposures of 250,000-490,000 ng/m³ were associated with lung cancer. Among pigment workers, an SMR of 190 was determined for workers exposed for more than 2 years at 500,000-2,000,000 ng/m³. Another plant yielded at risk workers with exposures of 500,000-1,500,000 ng/m³ for 6-9 years. In risk-associated chrome plating industries, men were exposed to mists of concentrated chromic acid. Risks were generally limited to men with greater than 15years work. A three month exposure to chromic acid mist found no excess. Only men working directly with or near the chromic acid baths were at risk. In an Italian plant, increased risk was found for men working at least 1 year near the baths with airborne levels of the acid mist at 60,000 ng/m³. In a Czech plant, air levels near the baths were above 400,000 ng/m³.

Basic Principles of Chromium Toxicology

Chromium is used in many different industrial and commercial practices and products including stainless steel, chrome plating, leather tanning, as an anti-rust agent, and in various dyes, paints and alloys as a pigment. Urban air concentrations from air pollution average 10-30 ng/m³ but can range up to 500 ng/m³. Soil typically contains 40-400 ppm (ng/mg) of chromium. Chromium in foods is present in the range of 20-520 ppb (ng/g) but varies widely with food type. Chromium is also present in tobacco, and is found in cigarette smoke.

There are two major "oxidation" states of chromium that are important for understanding the biology and chemistry of chromium, i.e., chromium(III) (Cr(III), Cr⁺³, trivalent chromium) and

chromium(VI) (Cr(VI), Cr+6, hexavalent chromium). Other oxidation states exist, and with the exception of chromium(0), which is neutral as chromium metal and is inert, these other oxidation forms are transient. Chromium(III) is the form of chromium found in nature, usually complexed with several other elements in ores or soils. It is also the form that is an essential trace element in humans and is found in virtually all plants and animals. Chromium is ubiquitous in the environment (principally as chromium(III)), and is found in water, air, soil and rock.

Chromium(VI) forms many different types of compounds, such as highly insoluble titanium and lead chromate, moderately soluble calcium chromate and zinc chromate, and the highly soluble sodium dichromate. Chromium(III) also forms many different types of compounds including “inorganic” forms such as chromium chloride, and “organic” or biological forms such as chromium picolinate and Low Molecular Weight Chromium complex (LMWCr). Each form exhibits different physico-chemical and biological properties.

The word “Chromate” or “chromate ion” or “chromate oxyanion” refers to chromium(VI) bound to four oxygen atoms (CrO_4^{2-}), and is the fully “dissolved” form of chromium(VI) which is able to cross cell membranes. The ability of chromate to dissolve is highly dependent on the initial form, i.e., the slightly soluble calcium, zinc and lead chromate releases chromate slowly, while the highly soluble sodium dichromate releases chromate readily in solution.

A major discovery was made by Mertz and co-workers at the U.S. Department of Agriculture in the 1950s, which was later confirmed by Schroeder, indicating that Cr(III) is an essential trace element in animals. Schroeder and colleagues showed that an absence of chromium in the diet led to glucose intolerance (a diabetic-like state) in animals. This was confirmed in a number of other laboratories, and chromium joined selenium, iron, zinc, calcium and other metals on the list of elements that are essential in the diet for normal health.

Later studies of total parenteral nutrition (TPN), receiving all nutrition from the artificial liquid diet in an intravenous bag, further confirmed that chromium is an essential element. Lack of chromium produces progressive glucose intolerance, a diabetic-like state that was not responsive to the addition of insulin, but immediately reversed after adding Cr(III) to the TPN. Cr(III) is now included in all TPN solutions. Every major nutritional expert, society, organization, and government and international panel has concluded that chromium(III) is an essential trace element in humans. Chromium(III) is added to many over-the-counter multi-vitamin and mineral supplements. Only one or two papers have attempted to say otherwise, at least one of which was written under financial inducement by a law firm with a vested interest in characterizing all Cr, including Cr(III), as a potential hazard (see preceding comments). Except for those few citations it is almost universally accepted that Cr(III) is an essential element.

Chromium(VI) exists at low levels in nature, but is produced industrially for commercial

purposes by oxidizing the chromium(III) to chromium(VI) using a process called roasting (strong oxidizing conditions and very high temperatures). Biological systems do not possess the oxidizing power needed to convert chromium(III) to chromium(VI) but chromium(VI) is readily chemically reduced to chromium(III) both in the environment and in humans and those of other animals and plants. The rapid and ready reduction of chromium(VI) to chromium(III), and chromium(III)'s essentiality in humans, is critical for understanding how chromium interacts with the human body.

As described above there is broad consensus that long-term occupational inhalation exposure to dusts of the intermediate soluble forms, calcium chromate, zinc chromate and lead chromate, or to concentrated chromic acid mists is associated with an increased risk of lung and other respiratory cancers. These historic occupational exposures to chromium (VI) that were associated with respiratory cancer were also characterized by irritation of skin by direct contact with very high concentrations of chromium dusts or acidic solutions. Nasal irritation has also been observed with high concentrations of airborne chromium(VI) and is a hallmark of occupational exposures. These overt dermal effects have not been observed below the current occupational thresholds. It is well-documented that these workers frequently had chromium-related skin lesions. Follow-up of these workers has demonstrated no increase in skin cancer or other cancers.

Animal studies show that certain intermediate soluble forms of chromium(VI) (calcium in its sintered form, zinc and lead chromate) rather than the soluble forms (like sodium chromate) or the highly insoluble forms (such as barium chromate), are potentially carcinogenic at the site of exposure. These studies also indicate that these compounds are not carcinogenic at any sites distal to the route of exposure. Even the compounds of intermediate solubility are only weakly carcinogenic in these tests, and only if the animals are exposed in a way that circumvents a normal exposure route. These include directly injecting chromium(VI) compounds into muscles, lungs or trachea, or implanting caged cholesterol pellets of chromium compounds into the animals' lungs. Positive results were only seen with the highest, oftentimes overtly toxic doses, and even under such conditions the tumor incidence was low.

The NTP toxicology studies on subchronic oral exposure are technically well done. The principle issue that needs to not be lost in the detail is that even the lowest dose was 14.3 mg/L (ppm) of sodium dichromate dehydrate) (5ppm of CrVI), a concentration sufficient to overwhelm oral and gastric reductive capacity. Despite these enormous doses most of the observations did not exhibit a consistent pattern of dose or duration dependence. It is also important to recognize that these enormous doses of CrVI actually serve to deliver an enormous amount of CrIII to the organs and cells in question. Remembering that CrIII is not without biological activity (acting as a co-factor in insulin action), it is entirely possible the some of the observed effects are due to the physiological effects of

massive CrIII overload.

Even at these high doses a consistent relationship between severity and dose was not observed. This implies the presence of effects caused by indirect mechanisms, likely chronic inflammation and/or tissue damage only observed at the highest doses. Urinalysis shows effects due to decreased water intake due to poor palatability of the yellow water. This dehydration alone is capable of rendering epithelial tissues more fragile. Changes in organ weights were only observed at doses above 500ppm (180 ppm Cr(VI)).

The results of the NTP assays are described repeatedly as “without clear dose-response relationship”. Indeed, minimal to mild histiocytic cellular infiltration was observed in all groups including the control animals. Even less toxicity was observed in mice compared to rats; in fact even at 1000 ppm for 3 months there was no evidence of any hepatotoxicity, only mild changes in some hematological indices that were attributed to changes in body weight (probably caused by massive CrIII overloading and its potential effects on insulin and glucose metabolism). What needs to be appreciated is that the lowest dose used in any of these studies is at or above saturation of gastric reductive capacity and yet still very little toxicity was observed except at the two highest doses (and often only at the one highest dose). At the lower end of these very high doses, only inconsistent observations were made and when “toxicity” was reported it was generally ranked minimal to mild. Only the index of Liver (fatty change) was ranked as moderate, but that was identical to the ranking of that same index in the Controls. The main point here is that these are massive doses and they are eliciting minimal effects. This important concept should not be lost in the mass of detailed results.

The NTP carcinogenesis studies in rats and mice show that there is no carcinogenic response except at the two highest doses that also produce chronic tissue damage at the sites of carcinogenicity. The dose-response is definitively non-linear, as is the absorption data described above. Given that the lowest dose is already above the reductive capacity of the oral cavity and stomach, these data provide strong evidence of the protective effects of the reductive capacity of blood components.

It should be noted that the NTP’s published report by Stout et al [Hexavalent Chromium is Carcinogenic to F344/N Rats and B6C3F1 Mice after Chronic Oral Exposure, Environmental Health Perspectives 117: 716, 2009] presents an inaccurate discussion of potential mechanism of action, drawn heavily from the 2006 Costa article, especially in criticizing the work of DeFlora. In point of fact, the results of the NTP assay give nearly definitive proof that the work of DeFlora is correct. Even the lowest dose of the NTP assay exceeds the reductive capacity of the oral cavity and upper digestive tract. Yet little toxicity and no carcinogenicity is observed except at the two highest doses.

The argument by Stout et al that the NTP doses were below gastric reduction-saturation,

based on a supra-linear (decreasing response with dose) rather than sub-linear (increasing response with dose) dose response is incorrect. If the doses were below saturation of reductive capacity, as the dose increased the ratio of unreduced CrVI to reduced CrVI (CrIII) in the stomach would increase (due to depletion of reductive capacity), and absorption would show an increasing rate of response (opposite of what was observed) because of an increased percentage of the total Cr that would be in the unreduced hexavalent state. Yet both absorption and toxicity exhibit a decreasing rate of response with dose in the NTP assay. This would actually be expected at supra-saturation doses: once the reductive capacity of the oral, digestive and blood components is exceeded, the organs receiving the highest amount of CrVI will sustain inflammatory tissue damage provoking tissue regeneration. It is unlikely that such tissue damage would display dose dependence since it only occurred at the two highest doses of the assay and it is a complex, disseminated biological response. It is likely then that a combination of three factors contribute to the high dose carcinogenic response: (i) tissue damage with regenerative cell proliferation, (ii) regenerative cell proliferation in the presence of macromolecular damage, and (iii) regenerative cell proliferation occurring in the presence of massive CrIII loading, which may affect insulin-dependent proliferative signaling. Thus, the scientific evidence does not support the conclusion that low level environmental exposure to chromium(VI) is associated with health effects of any kind.

This is explained by the complex chemico-biological interactions and pharmacodynamics of chromium and the ability of the body to rapidly and effectively reduce chromium(VI), the potentially toxic form of chromium, to chromium(III), the biologically essential form of chromium. At the physiological level, a broad scientific and governmental consensus has embraced the Physiological Reduction model first put forward by Dr. Silvio De Flora. At the cellular level, a broad scientific and governmental consensus has embraced the Uptake-reduction model of Dr. Karen Wetterhahn.

At the physiological level, De Flora and co-workers have produced a model of chromium reductive metabolism that explains the highly selective toxicity of chromium(VI) to the respiratory system at high doses. The extracellular components of tissues and body fluids possess tremendous capacity for reducing chromium(VI) to the essential element chromium(III). This is true for all three routes of exposure that are relevant to humans, i.e., inhalation (breathing of dusts, mists, vapors, etc.), oral ingestion (swallowing of chromium from dusts, mists, etc. in mucous and saliva, food and water intake) and dermal exposure (dusts, solids, mists and liquids on the skin). Dermal exposure to chromium(VI) does not yield significant absorption of chromium because the dermis acts as a physical barrier and also has high reduction potential. Similarly, oral ingestion of chromium(VI), even at high doses, is expected to result in rapid and near-complete (depending on the dose) reduction of chromium(VI) to chromium(III) with little chance for absorption of chromium(VI). Total absorption rarely

exceeds 10% by this route. The reason for this is that the saliva, the gastric juices, and then the intestinal fluids all have enormous reductive capacity for chromium(VI). Recent studies have shown that even concentrated solutions of Cr(VI) (1mM) are almost completely reduced to Cr(III) by gastric juice in less than one minute. Only very high doses of Cr(VI) will overwhelm this reductive capacity. Small amounts of Cr(VI) that temporarily escape reduction (because the rapid reductive rate constant is not perfectly instantaneous) will encounter the mucous barrier lining the respiratory and gastrointestinal tract and not get ready access to the cell surface. At extremely high doses some Cr(VI) may be absorbed as Cr(VI) (see below for further discussion), but will quickly be reduced to Cr(III) by interaction with huge quantities of reductive agents in the blood. As shown in many studies, it is extremely difficult to deliver a genotoxic dose of Cr(VI) to a tissue distal to the point of injection: except under conditions of massive dose the administered Cr(VI) arrives at distal tissues as Cr(III).

At the cellular level, the Wetterhahn uptake-reduction model describes the intracellular metabolism of chromium. Chromium(III) crosses cell membranes very poorly due to its structure and charge. Chromium(VI), on the other hand, is taken up by cells much better, since it has the same basic structure and charge as phosphate and sulfate. It should be noted that although it is often stated in review articles that chromium “readily crosses cell membranes”, only a fraction of the available chromium(VI) outside the cell (2-10% in most cases, depending on the system) actually crosses into cells even under idealized cell culture conditions. Once inside the cell, chromium(VI) is rapidly reduced, ultimately to chromium(III). In the process of this reduction, it goes through various intermediates, including chromium(V) and chromium(IV). It may also generate reactive oxygen species and other radical species but this is still under investigation, especially since it is now known that Cr(VI) is capable of direct oxidation of biological macromolecules. Following chromium(VI) uptake and reduction by cells, various forms of DNA damage can be measured, and chromium(VI) treatment of cells can increase mutations. Thus, the basic model for chromium carcinogenesis is that Cr(VI) reduction outside of cells is protective and Cr(VI) reduction inside the cell can lead to macromolecular binding, DNA damage and toxicity (explained in more detail below).

Chromium(III) crosses cell membranes only poorly due to its chemistry, and functions outside the cell by forming an amino acid complex, which is called either Low Molecular Weight Cr complex (LMWCr) or Chromodulin. This complex binds to the external surface of cells and enhances insulin signaling thereby helping to control glucose tolerance of our bodies and this mechanism is now well-established. A normal diet provides approximately 50-200 ug of Cr(III) per day and daily ingestion is required since Cr(III) is readily excreted from the blood into urine. Only 0.5-2% of available chromium(III) is absorbed.

Summary of Principles Derived from My Own Chromium Research

Not all chromium (VI) compounds are equally carcinogenic:

Trivalent chromium (CrIII) is nearly completely negative in virtually every animal bioassay and every in vitro assay it has been tested in. It is not recognized as either a mutagen or a carcinogen and in fact there is strong evidence that it functions as a necessary dietary essential element. Thus, this report will focus primarily on hexavalent chromium (CrVI) but will address trivalent chromium when necessary.

It is extremely important to understand that Cr(VI) exists in many different forms. There are completely soluble forms of Cr(VI), such as sodium and potassium chromate, which dissolve easily in water. Cr(VI) also exists in the form of solid particles which exhibit varying degrees of solubility. Some particulate forms are almost completely insoluble and can hardly dissolve in water at all (such as titanium chromate), some are mostly insoluble such that only a small amount dissolves in water (forms such as lead chromate), and there are moderately insoluble forms that dissolve to a moderate degree (forms such as calcium chromate and zinc chromate).

Early on it was recognized that both the epidemiological studies on chromate-exposed workers, and the in vivo (in the living animal) carcinogenesis assays of Cr (VI)-exposed animals, revealed that not all Cr (VI) compounds could be implicated as carcinogens. The epidemiological studies revealed that the site of action was almost exclusively limited to the respiratory tract and that the exposures were primarily through inhalation of either large quantities of particulate chromium (VI) compounds of limited solubility (moderately to highly insoluble) for long periods of time (as in chromium mining and chromate production), or chronic inhalation of a chromic acid mist (chrome platers). This is why the International Agency for Research on Cancer (IARC) has classified Cr(VI) as a carcinogen, "as it is encountered in the chromate production, chromate pigment production and chromium plating industries".

The early animal carcinogenesis data supported this concept. Less than half of the total animal experiments yielded a positive result, and the vast majority of animal experiments using soluble hexavalent chromium compounds were negative; only rarely and inconsistently was an extremely weak response observed using multiple repeated high dose exposure regimens. The early studies showed that only the particulate compounds of limited solubility were capable of tumor induction and only at the site of administration. This strongly suggested that there was something unique about the chromium particles of limited solubility such that long term, high dose exposure to them was weakly, but measurably, carcinogenic. It was this hypothesis that I first tested while still a postdoctoral fellow at

USC and then continued researching independently at GWUMC. These studies have helped us understand the differential carcinogenic potential of different Cr(VI) compounds.

The concepts emerging from my laboratory are summarized below. Using lead chromate as a prototypical particulate of limited solubility, we found that these particles are negatively charged and approximately 1 micron in diameter in the shape of hexagonal rods. Upon inhalation they are capable of deposition on any impact surface and most will adhere to the mucous lining of the respiratory tract. Most of the inhaled particles will be engulfed by specialized particle-scavenging cells called macrophages and removed through the mucous escalator to the mouth where they are spit out or swallowed. Some of the particles may adhere to cells of respiratory tract, and although they exhibit only limited solubility in water, the particles in contact with the cell surface begin to dissolve in the immediate micro-environment of the cell. Some of the particles are also internalized into lung fibroblasts or epithelial cells by a process of engulfment called phagocytosis. Only doses and durations of exposure high enough and long enough to evoke marked amounts of cell killing are capable of inducing mutagenesis and carcinogenesis. Most of the Cr(VI) oxyanions being dissolved from the particle outside the cell are quickly reduced to the trivalent form of chromium which is not readily absorbed by cells. The capacity of the extracellular milieu to reduce Cr(VI) to Cr(III) is markedly increased by the presence of ascorbate (vitamin C). In fact, the ability of ionic Cr(VI) produced by particle dissolution to get into cells and cause DNA damage can be nearly completely obviated by supplementing the cell growth medium with physiological concentrations of ascorbate. Some of the Cr(VI) released from the particles onto the surface of the cells will enter the cells through the anion transport system and undergo reductive metabolism to form pentavalent, tetravalent and ultimately trivalent species inside the cell. The principle intracellular reductants are ascorbate (vitamin C) and glutathione (GSH). Oxidative intermediates may also be generated in the process, but whether they are produced in normal cells at non-lethal doses, and what their role is in cellular responses to chromium, has not yet been fully established.

Modeling Particle Effects with Soluble Cr(VI): Evidence for a threshold: We have modeled the release of Cr(VI) ions from particles of limited solubility, in vitro, using soluble Cr(VI) compounds such as sodium chromate. Even in a cell culture medium, a completely “closed” system, which has a limited capacity to reduce Cr(VI) to Cr(III), we have found that continuous exposure for at least 18-24 hours was required to achieve intracellular levels of chromium comparable to what lead chromate particles could achieve. Comparable intracellular levels could also be achieved using much higher doses for shorter periods of time (the concentration x time ratio) but it was found that at any given dose, no additional uptake or toxicity could be achieved by treatment times exceeding 24 hours. Thus, no

cumulative uptake or damage would be expected for durations of exposure longer than 24 hours. The uptake of soluble Cr(VI) is extremely sensitive to the addition of reducing components in the culture medium and is nearly completely blocked by the addition of vitamin C due to the nearly instantaneous reduction of Cr(VI) to Cr(III). The amount of uptake in the presence of vitamin C is nearly identical to the low levels of uptake which occur when dosing with Cr(III). Thus, there is clear evidence of a “no effect level” and a threshold for chromium toxicity, simply based on the composition of the extracellular medium and prevention of Cr(VI) uptake.

We have also found clear evidence for an intracellular threshold for toxicity. For example, treatment of human lung fibroblasts with less than 1 μM sodium chromate [approximately 50 ppb of Cr(VI), or 50,000 ng/L of culture solution], for 24 hours, had absolutely no effect on cell survival, whereas 2, 4, 6 and 8 μM for 24 hours dose-dependently decreased cell survival from 100% to <5%, in one of the steepest survival/dose curves that we have ever observed. Thus, at the level of 50 ppb of pure hexavalent chromium in a closed system with limited reductive capacity (i.e. a petri dish), the chromium which enters the cell is effectively dealt with and completely detoxified. Given the enormous reductive capacity of human body fluids, one would have to start with a massive dose of soluble Cr(VI) to deliver 50 ppb (1 μM) directly to a cell.

We have proven that the DNA damaging, pseudo-mutagenic and neoplastic potential of Cr(VI) compounds occurs only at doses which overwhelm both the extracellular and intracellular protective mechanisms and cause forms of cell death known as apoptosis and terminal growth arrest. Over the past ten or so years, through research funded by the NIH, my laboratory has established the understanding that chromium carcinogenesis at the cellular level is likely to be a chronic process of selection of rare cells exhibiting gradually increasing resistance to cell death in the presence of chronic tissue damage due to long-term chronic exposure to toxic agents. Most recently, we have begun to identify the genes and molecular changes responsible for this rare conversion of lung cells to death resistance and my laboratory is at the forefront of elucidating the role of the ATM, AKT, ATR, PLK, ERK, p53 and other genes in the evolution of Cr(VI)-induced cancer.

We have also conducted and published studies on the role of DNA repair in chromium carcinogenesis. Contrary to the statements made by those who merely assume that inhibition of DNA will lead to increased mutagenesis, we and others have actually proven that the opposite is true of chromium. Under certain circumstances of exposure of cells to significant doses of Cr(VI), we do indeed find that the treatment can inhibit DNA repair, but we have also found that the loss of DNA repair leads to decreased mutagenesis. Chromium mutagenesis is actually suppressed in cell strains lacking specific DNA repair genes. This does not support the theory that Cr(VI) is carcinogenic by inhibition of DNA repair. Taken together, this indicates that at low dose exposures, no carcinogenic

response should be expected, and at high dose, long term exposure, at best only a weak carcinogenic response should be expected because the predominant effect of these doses is to induce cell death. This is entirely consistent with the epidemiological studies linking an increased incidence of lung cancer with high-dose, long-term exposure to highly insoluble chromate particulates or tissue-damaging chromic acid mists.

Why then did occupational chromium(VI) exposure increase risk of lung and other respiratory cancers? Firstly, workers were breathing in large concentrations of chromium-laden dusts, particularly those that penetrate to the deep lung (PM_{2.5} or less). Secondly, workers were chronically exposed to these dusts 8 hours per day, 5 days per week, 50 weeks per year, 15-40 years, such that there was a large daily and cumulative dose. Thirdly, exposure was to the intermediate soluble forms of chromium(VI) such that the particles allow slow dissolution of chromium(VI) to the cells surrounding the particle slowly over time. Studies of the lungs of chromium workers have shown massive accumulation of chromium(VI) dusts in these individuals, sometimes leading to chromium levels that could approach 10% of the weight of the lung. Because these intermediate soluble chromium(VI) particles dissolve slowly and are poorly cleared, they remain for very long periods of time.

It should be also be noted that although several epidemiological studies have suggested that the chromium-related risk of lung cancer in these workers may be distinguished over and above the risk from smoking, the vast majority of lung cancer cases were in chromium workers who were also smokers. Obviously, smoking is an additional potential source of chronic tissue damage.

Even particulate Cr(VI) compounds are weak transforming agents: The prediction made above is borne out in experiments showing that at least moderately toxic doses of Cr(VI) were required in order to cause a measurable mutagenic or neoplastic effect in several different types of cultured cells. These experiments further demonstrated the uniqueness of some of the particulate forms of Cr(VI) since only the particles of limited solubility were able to induce morphological or neoplastic transformation. These concepts have been further confirmed by other independent investigators as well. Even highly cytotoxic doses of completely soluble chromates (sodium chromate) or moderately soluble chromates (calcium chromate administered in its particulate form), were unable to induce morphological or neoplastic transformation. Thus, independent of dose or relative toxicity, the soluble chromium compounds were exceedingly inefficient as transforming agents. This is likely to be due, at least in part, to the extracellular and intracellular protective threshold mechanisms described above. However, it is also important to note that the relative potency of even the particulate chromates in causing cell transformation is extremely weak relative to a classic organic polycyclic aromatic hydrocarbon such as 3-methylcholanthrene. In experiments where completely non-toxic doses of 3-methylcholanthrene

would cause multiple cell transforming events in every culture dish, the chromate particles would barely induce 1 or 2 transforming events total in 20 culture dishes, and only at highly toxic concentrations.

Because a culture dish is a closed, non-dynamic system, it is relatively easy to overwhelm the extracellular reducing capacity of the culture medium and force the uptake of Cr(VI) by the cells. It is important to recognize, however, that cells also have a number of intracellular barriers to chromium toxicity as well. For example, during the reduction of Cr(VI), the newly formed Cr(III) is bound extensively by and to free amino acids, glutathione and small peptides and in this "liganded" state is dramatically less available for binding to critical macromolecules such as DNA. Also, many of the oxidative effects of Cr(VI) are reversed or blocked by antioxidants such as Vitamin E. If the intracellular barriers are also overwhelmed, reductive intermediates of Cr(VI) can cause a spectrum of DNA damage including single strand breaks, chromium-DNA adducts, DNA-protein crosslinks, DNA-DNA crosslinks, and chromosomal aberrations. Much of this genotoxicity can be prevented by pretreatment of cells with anti-oxidant vitamins such as ascorbate and tocopherol (Vitamin E). My laboratory has identified the chromium-induced DNA-DNA crosslink as one of the most damaging genotoxic lesions. We published a study showing that the presence of glutathione (a key intracellular protectant found in mM concentrations inside of cells) prevents formation of DNA-DNA crosslinks during chromium exposure. Another important consideration is that much of the DNA damage induced by chromium is repaired rapidly (for example all of the DNA breaks induced by toxic doses are repaired within 6 hours after treatment). We and others have shown that DNA repair may itself be partially inhibited by toxic Cr(VI) exposures. However, we have also shown that the loss of certain types of DNA repair leads to decreased chromium mutagenesis, not increased mutagenesis. Thus, not only are there profound barriers to chromium toxicity outside of cells in the body fluids, there are also barriers to chromium toxicity inside of cells as well.

Intersection of our work with Chromium Absorption and Distribution: The extracellular fluids of the human body possess enormous capacity to quickly reduce Cr(VI) to Cr(III), thus Cr(VI) is not considered a systemic toxicant except at extremely high doses administered as a single dose. There is no oxidizing environment in the human body capable of re-oxidizing Cr(III) to Cr(VI), thus Cr(III) is the final stable product. At doses which do not overwhelm the reductive capacity of a tissue or a system, the Cr(VI) is quickly reduced to Cr(III) with a half-life measured in seconds. Cr(III) is most likely absorbed by either passive or facilitated diffusion through the interstitial spaces surrounding the mucosal cells lining the tissue. After a single dose, absorbed Cr(III) will enter the bloodstream and transient increases in tissue chromium are followed by rapid elimination in the urine and accumulation of chromium in tissues cannot be detected. Chronic intake of high doses of Cr(III) will result in sustained increases in tissue levels of Cr(III), which quickly decrease as soon as the ingestion is

ceased. Human infants are born with high tissue levels of Cr(III) which decrease with aging, probably due to nutritional deficiencies.

When Cr(VI) is administered, most of it will be quickly reduced to Cr(III) by fluids associated with the treated tissue. Cr(VI) will apparently be absorbed better than Cr(III), but even at high doses, on average, less than 10% of Cr(VI) is absorbed. Another barrier to absorption of any Cr(VI) that temporarily escapes reduction is the mucous lining of the respiratory and gastrointestinal track. The mechanism of the increased absorption of the excess Cr(VI) is not known, but all of the possibilities would result in further reduction to Cr(III). One possibility is that it would be taken up by mucosal cells as Cr(VI), then reduced to Cr(III) intracellularly (and probably bound to peptides), as it is being transported across the mucosal cells for release into the blood. A second possibility is that the “excess” Cr(VI) is not really Cr(VI) at all, but a newly-formed type of Cr(III) which is absorbed better than free Cr(III). Recent published reports add significant support to the growing understanding that newly formed Cr(III) is chemically and reactively different than “aged” Cr(III), thus it is possible that newly formed Cr(III), produced by reduction from Cr(VI), may be better absorbed from the GI tract than aged Cr(III). This would further explain why total chromium absorption from the GI tract is greater with Cr(VI), but that the chromium arriving in the blood and distal tissues is Cr(III). It is also possible that at high doses, very small amounts of the excess Cr(VI) could theoretically be carried through the mucosal lining with the passive diffusion (absorption) of water. In this case the Cr(VI) would be absorbed by passing through the interstitial spaces and not by being transported into and across the cells of the mucosal lining. Regardless of how or in what form it crosses the mucosal lining, the absorbed chromium will make it into the blood stream where it will immediately encounter the enormous reducing capacity of red blood cells and the enormous protein binding capacity of the blood plasma. These principles are well established and recognized, having been reviewed by E.J O’Flaherty in 1995 in *Toxicology of Metals: Biochemical Aspects*.

Absorption by inhalation is limited by particle size, solubility, and phagocytic elimination by the 23 billion pulmonary macrophages (particle scavenging cells). The vast majority of inhaled particulates are efficiently removed by the muco-ciliary escalator. Highly soluble particles and aqueous mists (droplets) are reduced to Cr(III) by ascorbate, glutathione and other reducing equivalents present in lung lavage fluid in high concentrations. Highly insoluble particles, administered at high chronic exposure levels that overwhelm macrophage capacity, may persist in the respiratory tract and may contact the cells of the lung lining, leading to the events described above.

It is clear that we are faced with a unique situation in assessing the MOA of Cr(VI) at it relates to low-dose risk assessment. It is abundantly clear from all the science that the effects of Cr(VI) at the massive doses necessary to produce tissue toxicity and carcinogenesis in rodents, have no

bearing on the effects of low-dose, environmentally-relevant exposures. This is consistently borne out by epidemiological, animal and cell experimentation. This is especially pertinent in relation to whether or not Cr(VI) should be considered with a mutagenic MOA. I have spent more than 25 years studying the genotoxic properties of Cr(VI) and I have frequently contributed to the plethora of studies showing DNA damage and what we thought was associated mutagenesis. There is no doubt that Cr(VI) can be forced to be genotoxic and “mutagenic” under experimentally contrived systems using high doses that evoke major amounts of cell death. The question is, is Cr(VI) mutagenic at environmentally-relevant exposure levels? The growing consensus is that it is not.

In hindsight many of us “DNA damage and repair” scientists have come to appreciate several important factors: (i) DNA damage is only observed at very high dose that kill a lot of cells, (ii) Cr(VI) is at best a very weak “mutagen”, requiring very high doses that kill most cells and experimental “backflips” to select for survivors, and (iii) what we thought was “mutagenesis” is actually selection for stochastic cell survivors of massive toxic insult. Dr. Rossman’s group at NYU has shown that the base sequence of the genes used for mutation detection and selection is intact and that the changes in gene expression enabling selection are epigenetic, not mutagenic. Our group has shown that what we really selected for at toxic exposures are cells that are resistant to apoptosis, and Dr. Zhitkovich’s group at Brown has shown that the “mutant” cells were actually surviving cells that were selected for changes in specific forms of DNA repair. Again, this only occurs at doses that kill a lot of cells, not dissimilar to the high-dose rodent assays wherein tumors were only observed at doses that produced chronic and fairly severe tissue damage.

Regulatory agencies may be under certain historical precedents and pressures to deem Cr(VI) with a mutagenic mode of action simply because there are published studies that have “Cr(VI)” and “mutation” equated in the title (some of these papers are my own), but this decision would not be based on recent science. At high, tissue damaging doses, one can get tumors to form and those tumors will have mutations in specific genes because that is the molecular history of how that particular type cancer develops. It will not have any relation to chemically-specific mutations caused by Cr(VI) because Cr(VI) is an exceedingly poor mutagen. Even at the low end of the very high NTP doses there is no MOA because there is little or no toxicity, no mutagenicity, and no carcinogenesis. Extrapolating linearly from events observed at the two highest doses of the NTP assay, to anything close to reality for environmental exposure, is simply not scientific.

Summary: The carcinogenicity of Cr(VI) is limited to certain forms of Cr(VI) (highly insoluble particulates and mists of concentrated chromic acid) and require long-term exposure to high doses: Taken together, the experimental observations provide a mechanistic basis for understanding why the

epidemiological data shows that the carcinogenicity of Cr(VI) is limited to occupational settings. Only in occupational settings, and especially certain occupational settings prior to 1970, did the inhalation exposure reach sufficiently high levels of the intermediate soluble particles to induce a carcinogenic response. Indeed, several studies have suggested that even the risks for occupational chromium-related respiratory cancer decreased after simple industrial hygiene measures, such as wearing a mask, were implemented. Animal carcinogenesis studies show that completely soluble Cr(VI) is not carcinogenic by inhalation or ingestion.

It should be noted from the above discussion that Cr(VI) compounds are able to induce genotoxic damage in experimental animals when administered through routes that bypass or overwhelm the natural defense mechanisms, such as through intra-peritoneal injection, intra-tracheal instillation, or intra-gastric injection. For example, the massive reductive capacity of blood, which normally prevents adverse effects of Cr(VI) at a distance from the portal of entry, can only be overwhelmed by intra-peritoneal doses that exceed 50 mg per kg body weight. These protective mechanisms were acknowledged by the U.S. EPA when setting a maximum contaminant level goal (MCLG) of 100ug chromium/liter. USEPA reported that “the reduction of chromium(VI) to chromium(III) occurs in mammals”. The saliva and gastric juice in the upper alimentary tract of mammals, including humans, have a varied capability to reduce chromium(VI), with the gastric juice having notably high capacity. Likewise, the tracheo-bronchial tract and lungs also display high reducing capacity capable of handling the inhalation of droplets of aqueous chromium(VI) as alleged in this case. To the extent that chromium(VI) might survive these reduction environments, the blood plasma and red blood cells, as well as other organs/tissues such as the liver, are also reducing environments. Thus, the body's normal physiology provides detoxification for chromium(VI), which provides protection from the oral toxicity of chromium(VI).”

These conclusions are illustrative of the fact that Cr(VI) is poorly toxic and poses no carcinogenic risk by the oral route or by inhalation of droplets of water containing chromium(VI) [not including concentrated chromic acid mist]. Regarding human lethality, most humans survive even 10-15 grams of acute ingestion with the lethal oral dose of chromates is estimated at 50-70mg chromium(VI)/kg body weight. Studies in mice, rats, dogs and rabbits, wherein Cr(VI) was administered in drinking water at doses far in excess of drinking water standards for long periods of time, revealed no adverse effects. The USEPA cites that no adverse health effect was observed in a family drinking Cr(VI)-contaminated well water for 3 years. Likewise, in a 24 year period of follow-up, there was no increase in cancer in residents of Southern Mexico drinking groundwater containing 0.9 mg/liter total chromium. No increase of cancer was found in residents of Glasgow drinking water contaminated with chromium(VI) from chromate slag in soil containing 10,000mg total chromium, followed for 30 years.

Chromium contamination of drinking water in Woburn Massachusetts was put forward as working hypothesis to explain a purported excess of mortality to leukemia in children, but this hypothesis was shown to be incorrect by the same research group.

The WHO concluded that “there is insufficient evidence to implicate chromium as a causative agent of cancer in any organ other than the lung”. Likewise IARC concluded that “for cancers other than of the nasal and sinonasal cavity, no consistent pattern of cancer risk has been shown among workers exposed to chromium compounds”. The ATSDR report reached the same conclusion. The IARC Working Group reviewed the animal and human studies that show the existence of threshold mechanisms of Cr(VI) toxicity and carcinogenicity and “interpreted these findings as indicating mechanisms that limit the activity of chromium(VI) compound in vivo”. Likewise, in its prior toxicological review the USEPA concluded that “the body’s normal physiology provides detoxification for chromium(VI)” and the US Department of Health and Human Services indicated that these “mechanisms limit the bioavailability and attenuate the potential effects of chromium(VI) in vivo”.

Concluding Comments

There is a massive body of literature documenting what is referred to as the Uptake-Reduction model of chromium toxicity. Simply stated, Cr(III) (an essential element) is incapable of crossing cellular membranes to any significant extent. Its normal physiological function is to facilitate the interaction of insulin with insulin’s receptor on cell surface. Thus, the Cr(III) that we normally receive in large quantities from our diet, does not enter into cells. In contrast, the Cr(VI) oxyanion passes easily across cellular membranes because it is structurally similar to sulfate and phosphate and it piggybacks on the cell’s anion transport system. Cr(VI) itself is relatively un-reactive with other cellular macromolecules (like DNA or proteins) but once inside of cells, Cr(VI) gets metabolically reduced (thus the Uptake-Reduction Model) by intracellular reductants (ascorbate, glutathione, cysteine, etc.) to form potentially reactive intermediates Cr(V), Cr(IV) and ultimately Cr(III). Once it reaches its lowest energy state [Cr(III)] it cannot leave the cell as Cr(III) because it can’t cross the cell membrane. If it gets completely liganded (bound) to small peptides or amino acids, it can presumably leave the cell by passive diffusion. Under no circumstances would it be feasible or possible for Cr(III) inside of cells to be oxidized back to Cr(VI). Although this reaction can be forced to take place in a chemistry lab, the oxidizing power required to catalyze this reaction is completely incompatible with life and would destroy any cell near it.

It would be hard to exaggerate the importance of these fundamental concepts, which are uniformly accepted and embraced by both the scientific and regulatory communities. They explain why Cr(III)-piccolinate can be a \$200 million/year dietary supplement industry, whereas Cr(VI), in certain

forms and doses, can potentially be an occupational hazard. These concepts also provide a foundation for the importance of the human body's physiological extracellular reducing systems that mitigate the toxicologic potential of even large quantities of Cr(VI). Once extracellular Cr(VI) is reduced to Cr(III) outside of the cell, it becomes an essential nutrient and is incapable of causing any damage. It should be recognized that there is a vast literature describing the genotoxic and mutagenic effects of supra-threshold doses of Cr(VI) in experimental systems. There is also a vast literature on the effects of carcinogen doses of Cr(VI) on cell biology, gene expression and the process of malignant transformation. To the philosophical extent that scientists can "know" anything, much is known about Cr(VI) as an occupational carcinogen and therefore much can be ascertained about doses and exposures that represent true risks. It is not proper scientific methodology to ignore this knowledge and broadly state that the mechanism of chromium-induced lung cancer is either unknown or caused by oxidative stress or reactive oxygen species produced as a result of extracellular reduction of chromium. It should also be noted that compared to many organic mutagens (ie., certain specific PAH's), Cr(VI) is only weakly mutagenic, if mutagenic at all, and only at markedly toxic doses. It is inappropriate scientific methodology to simply state that Cr(VI) is mutagenic (without qualification) and therefore imply that any dose, no matter how small, will be a carcinogenic risk. Moreover, in several in vitro assays for neoplastic transformation, soluble Cr(VI) is actually unable to induce neoplastic transformation, even though the dose was high enough to damage DNA, presumably because its mechanism of mutation induction does contribute very well to transformation. There is no foundation for the belief, or the conclusions drawn from it, that any dose (concentration) of hexavalent chromium may meaningfully contribute to both risk and causation. This concept departs from accepted scientific methodology, which does not embrace a semantic or philosophic argument about whether a vanishingly small amount (down to a few atoms or molecules) of any substance can contribute to risk or causation. What is important to science and society is whether that risk or causation is meaningful, and this where experimental data provides appropriate information. The available data, much of which I have reviewed in this report, indicates that a very high dose is required for the carcinogenic effect of a limited number of forms of Cr(VI) as they can be encountered in the chromium industry.

At a minimum, methodologies and conclusions regarding Cr(VI) risk and causation have to be qualified with the critical concept of dose and detoxification thresholds. Most body compartments have enormous reductive power and rapidly reduce Cr(VI) to Cr(III), an essential nutritional element. Body fluids contain high concentrations of a number of reducing agents (including ascorbate), each of which will contribute independently and additively to Cr(VI) reduction to Cr(III). Many of these biological reducing agents are present in great excess over the concentrations of chromium that could be delivered by environmental exposure. It is not acceptable to not give adequate (or any) weight to the

extensive documentation of the reducing power of extracellular body fluids. To overlook this is to have overlooked the most basic principles of toxicology regarding detoxification.

Even if one presumes that a high enough dose of Cr(VI) actually gets to the cells of a particular tissue, one must also consider that there are a number of intracellular protective mechanisms which generate an intracellular threshold which must be breached by a high enough dose, before genotoxic endpoints will be reached. It is virtually inconceivable that such a high dose could be delivered to a cell with an environmental exposure, as is claimed by plaintiffs in this case. First of all, the cell's cytoplasm contains high (millimolar) concentrations of reducing agents, just like the extracellular environment. Most of the Cr(VI) which is reduced inside of the cell is converted to Cr(III) with its binding capacities quickly saturated with small molecules such as cysteine (and other amino acids) and small peptides such as the tri-peptide glutathione. In this liganded state, Cr(III) is virtually unreactive with additional macromolecules because its binding coefficient to protein is much higher than molecules such as DNA. Indeed, it is well known that binding of Cr(III) to peptides such as glutathione, prevents binding of the Cr(III) to DNA and also prevents the formation of several other DNA lesions.

Our current models for chromium genotoxicity require a dose of Cr(VI) high enough that some Cr(VI) can be reduced to its reactive intermediates in the immediate vicinity of the DNA, by a reductant which will not itself bind the intermediate and prevent it from interacting with the DNA. If such a dose is received, damage to DNA can occur. But that is not the end of the operative protective measures of the cell. My laboratory was the first to show that low levels of Cr(VI)-induced DNA damage trigger a classic DNA damage response (p53 induction) which stops the cell from dividing until it repairs the damage. This is now widely reproduced and accepted by the scientific community. Most types of Cr(VI)-induced DNA damage are effectively repaired within 8-24 hours after occurring. My laboratory was also the first to demonstrate that if the amount of DNA damage is too large to easily repair, the otherwise transient cell cycle arrest will convert to terminal growth arrest or apoptotic cell death. These are generally accepted mechanisms whereby a damaged cell will be eliminated and will no longer be a target for mutagenesis or neoplastic transformation. Thus, it is not appropriate for anyone to imply that if any dose, nor matter how small, of Cr(VI) reaches a cell, that cell is automatically a candidate for cancer initiation. This methodology is not supported by the peer-reviewed scientific literature and not accepted by the expert scientific community. It patently ignores the facts about the basic toxicology (physiological disposition and metabolism) of chromium and ventures into the realm of theoretical "biological plausibility". Likewise, it is also scientifically inappropriate to refer to mathematically-derived regulatory values as though they represent a biologically relevant threshold, above which genotoxic damage to a cell and development of cancer is nearly an inevitable outcome.

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