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S. HRG. 112–953

OVERSIGHT HEARING ON THE ENVIRONMENTAL PROTECTION AGENCY'S IMPLEMENTATION OF THE SAFE DRINKING WATER ACT'S UNREGULATED DRINKING WATER CONTAMINANTS PROGRAM

HEARING

BEFORE THE

COMMITTEE ON ENVIRONMENT AND PUBLIC WORKS UNITED STATES SENATE

ONE HUNDRED TWELFTH CONGRESS

FIRST SESSION

JULY 12, 2011

Printed for the use of the Committee on Environment and Public Works

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ONE HUNDRED TWELFTH CONGRESS
FIRST SESSION

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OVERSIGHT HEARING ON THE ENVIRONMENTAL PROTECTION AGENCY'S IMPLEMENTATION OF THE SAFE DRINKING WATER ACT'S UNREGULATED DRINKING WATER CONTAMINANTS PROGRAM

WEDNESDAY, JULY 12, 2011

U.S. SENATE,
COMMITTEE ON ENVIRONMENT AND PUBLIC WORKS,
Washington, DC.

The full committee met, pursuant to notice, at 10 a.m. in room 406, Dirksen Senate Office Building, Hon. Barbara Boxer (chairman of the full committee) presiding.

Present: Senators Boxer, Inhofe, Lautenberg, Cardin, Whitehouse, Udall, Boozman.

STATEMENT OF HON. BARBARA BOXER, U.S. SENATOR FROM THE STATE OF CALIFORNIA

Senator BOXER. Welcome, everybody. I have called this hearing to examine one of the most important Federal public health safeguards in our Country, the Safe Drinking Water Act. Everyone has a right to clean and safe drinking water, and it is essential to the health of our children and our families that drinking water be free from harmful chemicals and pollutants.

In order to ensure that enough is being done to protect our Nation from emerging contaminants, I, along with Representatives Waxman and Markey, asked the GAO to investigate the unregulated contaminant program. The report is being released today. I want to thank the GAO, the Government Accountability Office, for their work and welcome them here today, along with our Deputy Administrative. We welcome both.

The GAO investigation addresses the stunning fact that the EPA has not made a determination to regulate any new drinking water contaminants, with one very recent exception. And that failure to move goes back to 1996 when the law was last amended. This law was signed by Gerald Ford, who made a very eloquent statement about it. I think it is very important to remember that our landmark laws are bipartisan in nature, and why I think it is so important that we work across party.

This is what Gerald Ford said when he signed the Safe Drinking Water Act: "Nothing is more essential to the life of every single American than clean air and pure food and safe drinking water. There have been strong national programs to improve the quality of our air and the purity of our food." He said, "This bill, the Safe

Drinking Water Act, will provide us with the protection we need for drinking water."

And since I have mentioned Gerald Ford, I want to note the passing of this incredible former First Lady, Betty Ford, who I had the pleasure of meeting and following her career. I know I speak for everybody when we send our deepest condolences. Her life was a life that really impacted others because she had courage to speak about things that a lot of people were fearful to speak about, whether it was breast cancer or the challenges facing society or addiction to drugs and alcohol. I just think her kind of leadership, which she really did not seek the spotlight, had a tremendous impact. I wanted to make that note.

So when we talk about this report and why it is so important, I think the report is in the spirit of what Gerald Ford said, that we expect to have safe drinking water. In order to do that, we need to update our laws to make sure that we are not allowing certain contaminants in the water. And the GAO is very clear that EPA has to find what contaminants are dangerous and get them out of the water.

In 1996, Congress amended the Safe Drinking Water Act and directed EPA to use the best available science to create drinking water safeguards that would protect the most vulnerable, including infants, children and pregnant women. As the GAO report shows, the development of new standards for unregulated drinking water contaminants, such as perchlorate, were derailed in a process that failed to use the best available science.

We all say we want the best available science, Republicans, Democrats. We can just decide something is bad for you because it sound bad, has a scary name. We have to know the science behind it. And the fact is, we haven't seen any action since 1996 with this recent exception of perchlorate, for which I am very grateful. Because I have a bill with Senator Feinstein to regulate perchlorate. But it shouldn't be necessary to go to one contaminant at a time. The EPA has to act.

Scientific information has shown that certain emerging contaminants in our drinking water, such as perchlorate and chromium–6 could be harmful to children and families. Perchlorate, which is used in rocket fuel, fireworks and road flares, poses a threat to human health when found in drinking water. It impairs the function of the thyroid gland, which harms children's development and can result in decreased learning capacity. EPA needs to have a process that vigorously addresses these contaminants to help ensure the safety of our water.

GAO's report lays out a transparent and accountable framework that can help to ensure the EPA uses the best available science when creating drinking water protections for our communities and our families. Again, I am so glad that EPA is here today. And they are going to tell us about the implementation of the program. We are also going to hear GAO's testimony on the agency's need to use science to ensure that the Federal Government provides strong public health protections against drinking water contaminants.

And with that, Senator Inhofe.

STATEMENT OF HON. JAMES M. INHOFE, U.S. SENATOR FROM THE STATE OF OKLAHOMA

Senator INHOFE. Thank you, Madam Chairman. And let me tell you, I appreciate your remarks about Betty Ford. I happened to know her quite well. And you are right, she got into things, talking about alcoholism and she probably accomplished more than any other First Lady I can think of. They are celebrating her life today.

I think everyone in this room agrees that providing safe, clean, affordable drinking water is essential and should be a national priority. At the cornerstone of the Safe Drinking Water Act is the idea that we should be controlling those substances that pose risks to public health. Unfortunately, the system that EPA uses to determine health risks, the IRIS system, the Integrated Risk Information System, has decades-long issues in crafting risk assessments. The National Academy of Sciences has recently pointed out that IRIS assessments have suffered from lack of transparency and consistency and problems with evaluating studies and the weight of evidence. These problems continue to persist in the face of the NAS and Congress repeatedly imploring EPA to correct these issues.

Without having a good foundation and sound science, practices in all areas of EPA's regulatory system are suffering, including drinking water. Unbiased, high quality scientific analyses are important foundations for making drinking water decisions. When the risk assessment that EPA is producing are unable to maintain the highest possible standards for scientific quality and integrity, every decision that follows is called into question.

Senator Vitter and I are trying to get the EPA to explain how they plan to address the systematic concerns with IRIS that were raised by the recent NAS review of formaldehyde, a chemical we have decades of experience and information to draw upon. Now, if the IRIS review of a chemical that we have a great history on is so fundamentally flawed, it is hard to imagine how we will end up with good science on chemicals of emerging concern. I am looking forward to hearing from the EPA about how they plan to address these concerns.

As analytical techniques continue to improve, we are able to detect constituents at increasingly lower levels. This ever-increasing ability to detect will allow the numbers of chemicals in our water to increase infinitely. However, it is important that we do not associate any detection with risk. In nearly every case, the extremely low levels that we are detecting are well below the dosage that would affect public health. To be perfectly clear, exposure does not mean there is risk. This is just one more example of the importance of getting robust science to guide our policy decisions to help correctly communicate to the public what the risk associated with a particular contaminant is.

It is no surprise that nearly half of Americans are concerned about the quality of their drinking water when headlines and talking points are filled with alarmist stories of chemical detection with no information about what that means specifically to their health.

Furthermore, it makes no sense to continue to tighten drinking water standards and send drinking water down an aging and failing infrastructure system. EPA estimates that just to keep up with the current drinking water requirements over the next 20 years, el-

igible drinking water systems will need over $300 billion in infrastructure investment. We need to improve our Nation's drinking water facilities by authorizing and reauthorizing the State Revolving Loan Fund programs, both for drinking water as well as for wastewater.

I was extremely disappointed by EPA's cuts of almost $950 million to the SRF program. By making these cuts, EPA is increasing funding mandates on water providers throughout the Country. We can't expect our communities to continue to provide safe drinking water if they don't have the resources to do it.

This is something I have been talking about for such a long period of time. And I know my State of Oklahoma is not totally different than other States. But we have a lot of communities that just can't afford these things. When I was mayor of Tulsa, I have to say that the biggest problems, and I know Bob Perciasepe has heard me say this before, the biggest problems facing our cities are really not prostitution and crime and all, it is unfunded mandates. And that is something that we have to be familiar with and have to address.

And finally, Madam Chairman, I would like to request that the record of this hearing be kept open for two additional weeks, so that both outside groups and our witnesses here today have the opportunity to review the GAO report on unregulated contaminants, which has been unavailable to the public until now. So I think we may need to have the access to that, and then have a comment period.

I am looking forward to hearing from the witnesses today, especially Dr. Patierno, who will share his expertise in chromium–6, something that is very interesting to me concerning the problems that exist in my city of Norman, Oklahoma, Dr. Cotruvo, who can share his lengthy public health and drinking water experience, and Deputy Administrator Bob Perciasepe, whom we have gotten to know very well.

We are going to try to stay and maybe expedite this and get it all done throughout the second panel. Thank you, Madam Chairman.

[The prepared statement of Senator Inhofe follows:]

STATEMENT OF SENATOR JAMES M. INHOFE, U.S. SENATOR FROM THE STATE OF OKLAHOMA

Thank you, Madam Chairman, for taking the time today to continue our discussions about Federal drinking water programs. I know that everyone in this room agrees that providing clean, safe, affordable drinking water is essential, and should be a national priority.

At the cornerstone of the Safe Drinking Water Act is the idea that we should be controlling those substances that pose risks to public health. Unfortunately, the system that EPA uses to determine health risks, the Integrated Risk Information System (IRIS), has a decade's long issues in crafting risk assessments. The National Academy of Sciences (NAS) has recently pointed out that IRIS assessments have suffered from a lack of transparency, inconsistency, and problems with evaluating studies and the weight of evidence. These problems continue to persist in the face of NAS and Congress repeatedly imploring EPA to correct these issues. Without having a good foundation and sound science practices, all areas of EPA's regulatory system suffer, including drinking water.

Unbiased, high-quality, scientific analyses are important foundations for making drinking water decisions. When the risk assessments that EPA is producing are unable to maintain the highest possible standards for scientific quality and integrity, every decision that follows is called into question. Sen. Vitter and I are trying to

get EPA to explain how they plan to address the systematic concerns with IRIS that were raised in the recent NAS review of Formaldehyde, a chemical we have decades of experience and information to draw upon. If the IRIS review for a chemical that we have great history on is so fundamentally flawed, it is hard to imagine how we will end up with good science on chemicals of emerging concern. I am looking forward to hearing from EPA about how they plan to address these concerns soon.

As analytical techniques continue to improve, we are able to detect constituents at increasingly lower levels. This ever increasing ability to detect will allow the numbers of chemicals in our water to increase infinitely. However, it is important that we do not associate any detection with risk. In nearly every case, the extremely low levels we are detecting are well below the dosage that would affect public health. To be perfectly clear, exposure does not mean there is risk. This is just one more example of the importance of getting robust science to guide our policy decisions and to help correctly communicate to the public what the risk associated with a particular contaminant is. It is no surprise that nearly half of Americans are concerned about the quality of their drinking water when headlines and talking points are filled with alarmist stories of chemical detection with no information about what that means to their health.

Furthermore, it makes no sense to continue to tighten drinking water standards and send drinking water down an aging and failing infrastructure system. EPA estimates that just to keep up with the current drinking water requirements over the next 20 years, eligible drinking water systems will need over $300 billion in infrastructure investments. We need to improve our nation's drinking water facilities by reauthorizing the State Revolving Loan Fund programs, both for drinking water and waste water.

I was extremely disappointed by EPA's cuts of almost $950 million to the SRF program. By making these cuts, EPA is increasing unfunded mandates on water providers throughout the country. We cannot expect our communities to continue to provide safe drinking water if they do not have the resources to meet their infrastructure needs. We have the responsibility to ensure clean, safe, and affordable water for our country by providing the necessary resources to our states and local governments.

Finally, Madam Chairman, I would like to request that the record for this hearing be kept open for 2 weeks, so that both outside groups and our witnesses here today, have the opportunity to review this GAO report on Unregulated Contaminants, which has been unavailable to the public until now, and provide a more robust understanding of the recommendations and suggestions for the committee record.

I look forward to hearing from all of our witnesses today, especially Dr. Patierno, who will share his expertise in chromium–6, something of interest to me and to my constituents in Norman, OK, Dr. Cotruvo, who can share his lengthy public health and drinking water experience, and Deputy Administrator Perciasepe. Thank you.

Senator BOXER. Thank you so much. And without objection, we will keep the record open for 2 weeks so everyone can avail themselves of the report and ask questions of the GAO.

Senator Lautenberg, welcome.

STATEMENT OF HON. FRANK LAUTENBERG, U.S. SENATOR FROM THE STATE OF NEW JERSEY

Senator LAUTENBERG. Thank you very much, Madam Chairman, for holding this hearing.

Clean water is as fundamental to our health as breathing clean air. And since the Safe Drinking Water Act came about 37 years ago, we have made significant progress making sure the Nation's water supply is clean and safe. But we still have a lot of work to do.

Research shows that there are more than 140 chemicals in our drinking water that EPA does not regulate. In some parts of the Country, these chemicals include gasoline additives and pesticides. Other States, natural gas drillers inject diesel fuel and chemicals into the ground as part of the now more popular fracking process, and EPA is powerless to do anything about it because of a loophole

in the Safe Drinking Water Act that I and others on this Committee have fought to repeal.

In many ways, we are still being haunted by the neglect of the prior Administration, which repeatedly failed to set limits and strengthen regulations on pollution in the Nation's water supply. During the Bush years, each and every time that the EPA was given an opportunity to improve water safety, the agency chose not to act. Today, we are going to hear the results of a new Government Accountability Office investigation that sheds additional light on this negligence and misplaced priorities.

GAO's investigation shows how much the Bush administration looked the other way, instead of regulating dangerous chemicals like perchlorate, a toxic substance found in rocket fuel. Instead of letting agency scientists and the law drive regulatory decisions, political appointees, acting and meeting in secret, pushed the agency away from putting limits on perchlorate. Fortunately, under President Obama, EPA is moving in the right direction once again working on the public's behalf.

The agency is taking steps to set new limits on chemicals in our drinking water and doing more to determine the impact of natural gas drilling on our Country's water supply. The EPA is making good use of the tools under the Safe Drinking Water Act. But the law itself limits EPA's ability to protect the public's right to know. The Safe Drinking Water Act only allows EPA to regulate temporary monitoring of a small group of unregulated contaminants. So the public has no idea that they might be drinking water laden with unregulated contaminants like flame retardants, gasoline additives or other toxics.

Twenty-five years ago, I authored the Right to Know law on toxic chemical releases to make sure that people know about potentially hazardous substances in their communities. The public also has a right to know what is in their water. That is why I introduced the Drinking Water Right to Know Act. My bill would require a targeted increase in monitoring for unregulated pollutants that could be hazardous.

In addition, the bill would require EPA to make information on contaminants in drinking water more readily available online and in plain English. More information on contaminants will empower citizens and help government to make better decisions on pollutants in the water supply.

So I look forward to hearing from our witnesses about how we can work together to make sure that every person has clean, safe water to drink when they turn on the tap. Thank you, Madam Chairman.

Senator BOXER. Thank you very much.

Senator Boozman.

STATEMENT OF HON. JOHN BOOZMAN,
U.S. SENATOR FROM THE STATE OF ARKANSAS

Senator BOOZMAN. I will have a statement for the record. Thank you.

[The prepared statement of Senator Boozman was not submitted at time of print.]

Senator BOXER. Thank you.

Senator Cardin.

STATEMENT OF HON. BENJAMIN CARDIN, U.S. SENATOR FROM THE STATE OF MARYLAND

Senator CARDIN. Madam Chairman, I am going to put my full statement in the record, and I do want to welcome our witnesses. I just want to underscore a point that has been said, and that is Americans expect when they turn their taps on they are going to get clean water. And we have to make sure that in fact becomes, maintains reality.

The way that unregulated contaminants are handled raises concern. We know we review them every 5 years, but to move it to regulation has been extremely cumbersome and difficult. Some of our States have acted because the Federal Government has not been able to act. I am not sure that is the substitute for the Federal Government acting. To me, this is one of our principal responsibilities, to make sure that we have safe and clean water for the people of this Nation.

I look forward to this hearing.

[The prepared statement of Senator Cardin follows:]

STATEMENT OF HON. BENJAMIN L. CARDIN, U.S. SENATOR FROM THE STATE OF MARYLAND

Madame Chairman, thank you.

Readily available, safe drinking water is an expectation most people in our Nation have come to take for granted.

The Safe Drinking Water Act Amendments of 1996 contain a provision that requires the EPA to evaluate chemicals in our drinking water and to regulate them as necessary. In its recent preliminary regulatory determination, however, the EPA has decided that it will NOT regulate 11 new chemicals found in our Nation's tap water.

One such contaminant is perchlorate—a chemical found in fireworks, explosives, and rocket motors. The chemical has been found in the drinking water of at least 35 states and the District of Columbia. Perchlorate has been found in breast milk.

It is known that perchlorate affects the ability of the thyroid gland to take up iodine. Iodine is needed to make thyroid hormones that regulate many body functions after they are released into the blood. Children are more likely to be affected by perchlorate than adults because thyroid hormones are essential for normal growth and development.

Despite the known science on the hazards of perchlorate, EPA has failed to establish a safety standard for perchlorate in drinking water.

Lacking Federal leadership, several states, including Maryland have adopted their own standards for perchlorate in drinking water. These standards range from 2 parts-per-billion in Massachusetts to 6 parts-per-billion in California. In 2002, my State of Maryland established a 1 ppb advisory level for perchlorate. There are two communities in Maryland that exceeded this advisory level and have since installed treatment facilities: the city of Aberdeen in Harford County and the Sherwood Forest Mobile Home Park in Cecil County.

Given the disparities in the advisory levels and standards for perchlorate from one State to another, our constituents may be confused and concerned that there is no National standard for perchlorate in their water given the risks, in particular to pregnant mothers and young children, of low-level exposure to perchlorate.

My concerns about perchlorate, particularly the EPA's failure to provide a regulation requiring monitoring of and notification for perchlorate in drinking water have led me to co-sponsor two bills that you, Madame Chairman have offered: S. 24, the "Perchlorate Monitoring and Right-to-Know Act of 2007" and S. 150. the "Protecting Pregnant Women and Children From Perchlorate Act of 2007"

A similar lack of action has existed for trichloroethylene or TCE: Even after a draft EPA Risk Assessment found that TCE was as much as 40 times more carcinogenic than previously thought, rather than setting a more protective standard for TCE in drinking water, the EPA called for more study.

Given the EPA's recent history of not particularly timely development of new regulations, I am concerned that it may be several years before this new information might be incorporated into new regulatory standards.

Existing scientific studies strongly suggest that the EPA should be acting expeditiously on developing regulations to limit exposure to perchlorate and TCE. It is unfortunate, that as with other environmental matters, the EPA has dragged its feet when the health of our Nation is at risk.

I look forward to the testimony from today's witnesses in helping to clarify this issue. There is scientific justification for the EPA to act quickly on these chemicals in our drinking water. If EPA cannot act, we must. Our country deserves better.

Thank you Madame Chairman.

Senator BOXER. Thank you very much.

We will now turn to our panel. I think we are going to hear from the Government Accountability Office first, so that Hon. Deputy Administrator can respond to their findings. So Mr. Trimble, we welcome you and we thank you for all your heard work. We look forward to your presentation. You can summarize in 5 minutes, and if we have further questions, we will ask them.

STATEMENT OF DAVID C. TRIMBLE, DIRECTOR, NATURAL RESOURCES AND ENVIRONMENT, GOVERNMENT ACCOUNTABILITY OFFICE

Mr. TRIMBLE. I am pleased to be here today to discuss our report being released today on EPA's program for regulating additional drinking water contaminants. We evaluated EPA's implementation of the 1996 amendments to the Safe Drinking Water Act, and reviewed the process and findings EPA used in making its 2008 preliminary decision to not regulate perchlorate.

We found that systemic limitations in EPA's implementation of the requirements for determining whether additional drinking water contaminants warrant regulation have impeded the agency's process in assuring the public of safe drinking water. These limitations start with the contaminants that EPA chooses to assess for regulation. Specifically, EPA's selection of contaminants for regulatory determination in 2003 and 2008 was driven by data availability, not consideration of greatest public health concern, as specified in the statute. In fact, an EPA official described the agency's regulatory determinations as addressing the low-hanging fruit.

Moreover, EPA has not been effective in obtaining occurrence of health effects data on contaminants, both of which are essential for regulatory determination. This program to test drinking water for unregulated contaminants has not tested as many contaminants as it could have. Also, in many cases, the testing methods have not been sufficiently robust to detect the contaminants at the level of public health concern, reducing the credibility of EPA's determinations. Information on the health effects of contaminants has also lagged due to longstanding challenges in the IRIS program and coordination issues.

Importantly, EPA has not developed regulations, policies or guidance for interpreting the amendments' broad statutory criteria for selecting contaminants and making decisions on them. In the absence of guidance, EPA has been informally applying a policy that contaminants need to occur in drinking water on a national scale to warrant regulation.

However, a national occurrence threshold is not required by the Safe Drinking Water Act, which in fact provides relief to public

water systems for monitoring when a contaminant occurs in certain areas but not in others. We have concerns about EPA implementing a critical policy which interprets the Act and has important implications for the safety of public drinking water when this policy has neither been defined nor subjected to public review.

Also, the credibility of some of the EPA's regulatory determinations is limited by a lack of transparency, clarity and consistency. For example, EPA states unequivocally in its regulatory determination support document that there is no data to indicate children are more sensitive to manganese than adults, but in its health effects support document discusses studies that do associate manganese in drinking water with learning disabilities in children. Our report details numerous other limitations that reduce the credibility of EPA's regulatory determinations, including making determinations on the basis of outdated and incomplete occurrence data, and the Office of Water's lack of protocol and methods for assessing drinking water health risks for children and other sensitive sub-populations, 15 years after the 1996 amendments required EPA to consider effects on such populations.

Regarding perchlorate, we found that the process and analyses EPA relied on to support its preliminary determination to not regulate were atypical, lacked transparency and limited the agency's independence in developing and communicating its scientific findings. While EPA's 2011 decision to regulate perchlorate included an analysis on the potential health impact on children, addressing key shortcomings in the 2008 decision, it is not clear at this time whether EPA has committed to do such analyses in the future.

We made 17 recommendations to support the development of transparent, clear and consistent regulatory determinations. An overarching recommendation is that EPA develop policies and guidance clearly articulating the agency's interpretation of the Act's statutory criteria as well as eight additional recommendations identifying specific components of this guidance.

EPA's response to this recommendation was that it was not practical to establish policies or guidance, citing various complexities. However, the complexities cited would argue for rather than against the need to develop guidance for staff in applying the criteria.

EPA also said it did not support developing policies or guidance because under the statutory criteria, it is the Administrator's judgment as to whether regulation presents a meaningful opportunity for health risk reduction after considering information presented by agency staff. The statutory criteria are so broadly stated that they could potentially be interpreted to support regulating all the contaminants on the candidate list, some of them or none of them.

It is precisely for these reasons that we believe it is essential for EPA staff to have sufficient guidance on applying the criteria consistently and transparently, so that the Administrator's judgment can be based on sound and consistent information. Without such guidance, the basis for EPA's determinations and the quality of their support can fluctuate over time as a result of, among other reasons, changes in agency leadership and staff.

In large measure, EPA's response to our recommendations essentially endorses conducting business as usual, a response that does

not acknowledge the scope and significance of the implementation limitations we identified.

I would be happy to respond to any questions that you or other members of the Committee may have.

[The prepared statement of Mr. Trimble follows:]

United States Government Accountability Office

GAO

Testimony
Before the Committee on Environment and Public Works, U.S. Senate

For Release on Delivery
Expected at 10:00 a.m. EDT
Tuesday, July 12, 2011

SAFE DRINKING WATER ACT

Improvements in Implementation Are Needed to Better Assure the Public of Safe Drinking Water

Statement of David C. Trimble, Director
Natural Resources and Environment

U.S. Government Accountability Office
GAO 90 YEARS 1921-2011
ACCOUNTABILITY * INTEGRITY * RELIABILITY

GAO-11-803T

Madam Chairman Boxer, Ranking Member Inhofe, and Members of the Committee:

I am pleased to be here today to discuss highlights of GAO's report on the Environmental Protection Agency's (EPA) implementation of requirements for determining whether additional drinking water contaminants warrant regulation. As you know, the number of potential drinking water contaminants is vast—as many as tens of thousands of chemicals may be used across the country, and EPA has identified more than 6,000 chemicals that it considers to be the most likely source of human or environmental exposure. The potential health effects of exposure to most of these chemicals, and the extent of their occurrence in drinking water, are unknown. Under 1996 amendments to the Safe Drinking Water Act, every 5 years EPA is to determine for at least five contaminants whether regulation is warranted, considering those that present the greatest public health concern. EPA issued final regulatory determinations in 2003 and 2008 on a total of 20 contaminants, deciding in each case not to regulate. In fact, EPA did not recommend any new contaminants for regulation until February 2011, when it reversed its controversial 2008 preliminary decision to not regulate perchlorate, an ingredient in rocket fuel and other products.

This statement summarizes our report being released today that (1) evaluates the extent to which EPA's implementation of the 1996 amendments has helped assure the public of safe drinking water and (2) reviews the process and scientific analyses EPA used to develop the 2008 preliminary decision to not regulate perchlorate.[1] In preparing this testimony, we relied on our work supporting the accompanying report, which contains a detailed description of our scope and methodology. All of the work for this report was performed in accordance with generally accepted government auditing standards.

[1]GAO, *Safe Drinking Water Act: EPA Should Improve Implementation of Requirements on Whether to Regulate Additional Contaminants*, GAO-11-254 (Washington, D.C.: May 27, 2011).

Systemic Limitations in EPA's Implementation of Requirements for Determining Whether to Regulate Additional Contaminants Have Impeded Progress in Helping Assure the Public of Safe Drinking Water

As detailed in our report, we found the following concerning the extent to which EPA's implementation of the 1996 amendments has helped assure the public of safe drinking water.

EPA Has Neither Identified the Drinking Water Contaminants of Greatest Public Health Concern Nor Fully Used Its Authority to Obtain Data for Making Regulatory Determinations

EPA has not effectively implemented the 1996 amendments' requirement to consider, for regulatory determinations, contaminants that present the greatest public health concern. The contaminant candidate list[2] that the amendments require EPA to develop every 5 years represents one level of prioritization as EPA selects from a larger universe those contaminants the agency believes warrant consideration for regulation. However, EPA officials told us that its Office of Water, which has primary responsibility for implementing the requirements of the Safe Drinking Water Act, has not (1) further ranked or otherwise prioritized the contaminants on the list on the basis of public health concern or (2) prioritized contaminants on the basis of public health concern when selecting them for regulatory determinations. In fact, for 16 of the 20 regulatory determinations made through January 2011, EPA based its decisions to not regulate on its assessment that public exposure to these drinking water contaminants was minimal—that is, there was limited or no occurrence of them in public drinking water systems. An EPA official described these determinations as addressing the "low hanging fruit"—rather than the contaminants of greatest public health concern. Overall, data availability—not

[2]The 1996 amendments require that EPA identify and publish a list every 5 years of unregulated contaminants that may require regulation; the list is called the contaminant candidate list.

consideration of greatest public health concern—has been the primary driver of EPA's selection of contaminants for regulatory determinations.

To assess unregulated contaminants against the statutory criteria, EPA needs sufficient information on both (1) the occurrence of these contaminants in drinking water—called occurrence data—to assess the population potentially being exposed and the levels of that exposure and (2) the human health effects that may result from exposure to the contaminants in drinking water. EPA has made some progress in developing the occurrence and health effects data it needs, but for many contaminants EPA lacks sufficient occurrence and health effects data to support regulatory determinations, which continues to limit its ability to make these decisions. Specifically, in finalizing its current candidate list comprising 116 contaminants, EPA indicated that the agency lacked sufficient occurrence or health effects data, or both, for making regulatory determinations for at least 100 of the contaminants. Moreover, in 2009 EPA's Science Advisory Board recommended that the agency further prioritize among the contaminants on the candidate list because the list was too large, noting that prioritizing the contaminants on the list would help the agency meet its goal of selecting contaminants for regulatory determinations that "have the greatest opportunity to improve the safety of drinking water and protect public health."

In addition, in its testing program for unregulated contaminants—which can provide key occurrence data to inform regulatory determinations—EPA has fallen short in both the number of contaminants tested and the utility of the data provided because of management decisions and program delays. For example, despite having the authority to require testing for up to 30 drinking water contaminants in each 5-year cycle, in implementing the first two cycles of the testing program, EPA required that only 51 contaminants be tested—thereby not availing itself of its authority to obtain occurrence data for 9 additional contaminants. Moreover, in some cases, the occurrence data EPA used to support its regulatory determinations were based on testing (analytic) methods that were not sufficiently sensitive to identify the presence of contaminants at EPA's health reference level—the level that EPA uses in assessing whether to regulate specific contaminants.[3] For 9 of the 20 contaminants

[3]The health reference level is the estimated level of exposure to a contaminant in drinking water below which adverse health effects are not likely.

for which EPA made regulatory determinations in 2003 and 2008, the minimum reporting level—the lowest level of a contaminant at which detections can be reported under testing protocols—exceeded EPA's health reference level. For example, for dieldrin—an insecticide banned by EPA for all uses in 1987 because of concerns about harm to human health and its ability to persist in the environment for decades—the agency relied on testing data obtained using minimum reporting levels ranging from 10 to 2,200 times higher than EPA's health reference level.[4] EPA reported in its regulatory determination documents for dieldrin that it was detected in 0.06 percent of samples.[5] However, in subsequent testing of source water for drinking water wells using more sensitive tests with minimum reporting levels near and below EPA's health reference level, the U.S. Geological Survey (USGS) detected dieldrin in 3.1 percent of public well samples. Importantly, nearly all of USGS's detections were at levels above EPA's health reference level. USGS was able to detect dieldrin—and determine its presence above EPA's level of public health concern—in these groundwater well samples because it used a lower minimum reporting level for its testing than EPA used for its regulatory determinations. This is significant because, as USGS has reported, when a reporting level exceeds a health benchmark, a contaminant may be present at a concentration greater than the health benchmark but remain undetected, resulting in greater uncertainty in evaluating the contaminant concentration in the context of public health. EPA's testing program obtains data using minimum reporting levels that are often higher than those used by the USGS in its National Water Quality Assessment Program—ranging from 2 to more than 600 times higher.

In addition, the lack of timely health assessment data on drinking water contaminants continues to limit EPA's ability to make regulatory determinations. As a result of long-standing productivity problems in EPA's Integrated Risk Information System (IRIS) program—managed by the Office of Research and Development—EPA has not been able to keep its existing chemical toxicity assessments current or to complete

[4]EPA did not disclose that the data presented were not sufficiently sensitive to detect occurrence at the agency's health reference level.

[5]In this testimony, as in our report, we refer to *Federal Register* notices regarding EPA's regulatory determinations (notices) and EPA's regulatory determination support documents individually and collectively, as appropriate. When referring to these documents collectively, we use the term "regulatory determination documents."

assessments of the most important chemicals of concern.[6, 7] For example, from 1998 through 2008, the Office of Water lacked current IRIS assessments[8] or other sufficient health information for 24 chemical contaminants on the candidate lists, and the Office of Research and Development completed assessments for only 2 of the 24. Moreover, the Office of Water's current needs for health effects information for contaminants on the current candidate list have roughly doubled—when publishing the third candidate list in 2009, EPA identified health effects information gaps for 44 of the 104 chemicals on the list. Importantly, most of these contaminants with information gaps (1) are not on the IRIS agenda (i.e., assessments are neither under way nor planned) and (2) have not been identified by the Office of Water as priorities for IRIS assessments.

EPA Lacks Policies or Guidance on Applying the Broad Statutory Criteria for Selecting Contaminants for Regulatory Determinations and Making the Determinations

The Safe Drinking Water Act requires EPA to select contaminants for regulatory determinations that present the greatest public health concern. However, EPA has not defined the characteristics of contaminants of greatest public health concern or developed a process for prioritizing the contaminants on its candidate list for regulatory determination on this basis. As a result, EPA lacks criteria and a process for identifying those contaminants on its candidate list that pose the greatest public health concern.

Moreover, under the act, in selecting contaminants that present the greatest public health concern, EPA is to consider the effect of these contaminants on subpopulations at greater risk of adverse health effects from exposure to drinking water contaminants. In addition, EPA has

[6]See GAO, *Chemical Assessments: Low Productivity and New Interagency Review Process Limit the Usefulness and Credibility of EPA's Integrated Risk Information System*, GAO-08-440 (Washington, D.C.: Mar. 7, 2008).

[7]In January 2009 we added transforming EPA's processes for assessing and controlling toxic chemicals as a high-risk area in our report—updated in February 2011—on governmentwide high-risk areas requiring increased attention by executive agencies and Congress. See GAO, *High-Risk Series: An Update*, GAO-09-271 (Washington, D.C.: January 2009); and *High Risk Series: An Update*, GAO-11-278 (Washington, D.C.: February 2011).

[8]IRIS assessments provide EPA's toxicity assessments of contaminants that may cause cancer and those that may cause neurological or other noncancer effects, or both. EPA uses IRIS or comparable toxicity assessments to develop health reference levels for the drinking water contaminants.

stated that in making regulatory determinations, the act requires the agency to consider the effects the contaminants have on sensitive subpopulations, such as infants, children, those with kidney or liver diseases or weakened immune systems, and the elderly. Children, for example, represent a sensitive subpopulation because they may be more highly exposed to toxic substances in drinking water and at greater risk of adverse health effects than adults as a result of consuming more water per unit of body weight than adults. Children may also have increased susceptibility following exposure to drinking water contaminants because they continue to develop both behaviorally and physiologically throughout childhood. Furthermore, in 1995, EPA published its *Policy on Evaluating Health Risks to Children*, which states that the agency will "consider the risks to infants and children consistently and explicitly as a part of risk assessments generated during its decision making process," and to "the degree permitted by available data in each case, the Agency will develop a separate assessment of risks to infants and children or state clearly why this is not done." In 2006, EPA developed a general guidance document for all EPA program offices on implementing its 1995 children's health policy, as well as several technical guidance documents that could help the Office of Water develop its own guidance specific to assessing the sensitivity of children to drinking water contaminants.

Notwithstanding the requirements of the Safe Drinking Water Act and EPA's 1995 children's health policy, the Office of Water did not implement a specific approach for considering children's health in developing its 2003 and 2008 regulatory determinations. In addition, the Office of Water has not developed guidance for its staff on when and how to analyze the effects of drinking water contaminants on children—or other sensitive subpopulations—for the purposes of identifying the drinking water contaminants of greatest concern on which to make regulatory determinations and to ensure it consistently and explicitly considers risks to children in making these determinations, such as by developing separate health reference levels for children. While EPA identified children as a sensitive population in 11 of the 20 regulatory determinations it completed in 2003 and 2008, Office of Water officials confirmed that for these 20 determinations, EPA did not develop separate health reference levels for children or make adjustments to its health assessments.

The 1996 amendments also provide three broad criteria for EPA to use in making regulatory determinations, all of which must be met for EPA to determine that regulation is warranted. Notably, two of the criteria are so broadly stated that they could potentially be interpreted so as to lead to

regulating all of the contaminants on candidate lists, some of them, or none of them. Specifically, the second statutory criterion—that a contaminant is "known to occur or there is a substantial likelihood that the contaminant will occur in public water systems with a frequency and at levels of public health concern"—is susceptible to varying interpretations. For example, different people may reasonably have differing views on the frequency and levels of occurrence that represent a public health concern. The third criterion—that regulation of the contaminant presents "in the sole judgment of the Administrator . . . a meaningful opportunity for health risk reduction"—is expressly discretionary, and similarly open to differing interpretations.

Importantly, the Office of Water has not developed policies or guidance to help EPA staff apply these broad criteria. Guidance that might help EPA staff apply the criteria transparently and consistently could, among other things, (1) define or set thresholds or parameters for assessing whether a contaminant occurs, or is substantially likely to occur, in public water systems with a frequency and at levels of public health concern and (2) provide factors or characteristics of situations that would present meaningful opportunities for health risk reduction. We note that such guidance could also serve as the basis for an internal review mechanism to help EPA ensure consistent implementation of the statutory criteria. Office of Water officials could not describe examples of what would meet the three criteria beyond stating that "there are no bright lines" and that they would "know it when we see it." Without clarifying guidance, EPA's regulatory determinations lack transparency, and EPA is at risk of making inconsistent determinations, undermining the program's credibility and the agency's ability to assure the public of safe drinking water.

In the absence of regulations or guidance for applying the broad statutory criteria, EPA appears to apply an informal policy that contaminants warranting regulation should occur in public water systems on a "national" scale. For example, documents supporting EPA's 2003 regulatory determinations state that the consideration of geographic distribution "is important because the agency is charged with developing national regulations, and it may not be appropriate to develop [national primary drinking water regulations] for regional or local contamination problems." In addition, some EPA officials serving on regulatory determination work groups told us that a contaminant must occur "nationally" to warrant a determination to regulate. Notably, however, the Safe Drinking Water Act does not require that contaminants be found in public water systems on a national basis for an Administrator to find a meaningful opportunity for health risk reduction. In fact, other parts of the statute provide for relief

from monitoring and flexibilities for instances in which a contaminant occurs in certain areas but not in others. Moreover, there is nothing in the act's committee reports suggesting that a contaminant need occur nationally to support a decision to regulate. Without EPA guidance providing a definition or parameters, an informal "national occurrence" standard is open to shifting interpretations, potentially affecting the consistency and credibility of EPA's decision making. To the extent EPA is informally applying an unspecified national occurrence requirement for contaminants to be evaluated as occurring "with a frequency and at levels of public health concern," EPA is implementing a critical policy and interpretation of the Safe Drinking Water Act that has neither been defined nor subjected to public review.

Further, regarding the third statutory criterion, EPA has not articulated guidelines or thresholds for how it is to assess whether regulating a specific contaminant would provide a meaningful opportunity for health risk reduction. The absence of guidelines on what scenario or scenarios might illustrate "a meaningful opportunity for health risk reduction" increases the potential for inconsistent decision making and reduces the decisions' transparency.

In addition, EPA has not developed any guidance on the circumstances that would trigger a re-evaluation of a prior decision to not regulate or the process the agency would use in conducting a re-evaluation. In at least one instance—1,1,2,2-tetrachloroethane—an updated IRIS assessment became available after EPA's determination to not regulate, but the agency has not announced whether it will reconsider the determination. In addition, as discussed in the following section, the credibility of some of EPA's regulatory determinations was limited. As we reported, we believe EPA should consider whether it needs to re-evaluate any of its past determinations to not regulate in light of the systemic and individual shortcomings we identified. In the absence of policies or guidance that identifies the circumstances that would trigger such re-evaluations and the process the agency would use in conducting them, it is not clear how and when such re-evaluations would occur.

The Credibility of Some of EPA's Regulatory Determinations As Presented in *Federal Register* Notices and Support Documents Is Limited by a Lack of Transparency, Clarity, and Consistency

The Safe Drinking Water Act requires EPA to ensure that, in its regulatory determinations, among other things, the presentation of information on public health effects is comprehensive, informative, and understandable. In addition, to the extent that EPA's regulatory determination notices and key support documents are transparent, clear, and consistent regarding the occurrence and health effects data the agency relied on, the credibility of the determinations is enhanced. However, for the regulatory determinations that EPA has made to date, some of the notices and support documents lack these key qualities. For example, as reflected in the following excerpts from EPA's regulatory determination documents for manganese and boron, EPA's presentation of health effects information on some contaminants lacked clarity, consistency, and transparency.

- EPA's 2003 regulatory determination support document for manganese states unequivocally that there are "no data to indicate children are more sensitive to manganese than adults." However, EPA's 2003 health effects support document for manganese discusses studies that identify an association between exposure to manganese in drinking water and learning disabilities in children and concludes that additional studies are needed to investigate the possibility that children are more sensitive than adults.

- EPA's regulatory determination support document for manganese notes that infants and newborns may be potentially susceptible to manganese toxicity, but this key document does not disclose that newborns may be exposed to high levels of manganese from infant formula or that these high levels of manganese in formula can be magnified when it is reconstituted with manganese-contaminated water.

- In its regulatory support document for boron, the Office of Water (1) identified the primary adverse effects identified from studies of animals after chronic exposure to low doses of boron as generally involving the testes and the developing fetus and (2) stated that animal studies identify the developing fetus as "potentially sensitive to boron" and concluded that boron concentrations greater than the health reference level "might" have an effect on prenatal development. In contrast, the Office of Water's May 2008 *Drinking Water Health Advisory for Boron*—developed in conjunction with the regulatory determination and published just 2 months before the regulatory determination was issued—states that there are "compelling lines of evidence to suggest that the testicular morphological effects" reported in studies of animals are applicable to children. Also, the Office of

Water's health advisory was not limited to prenatal exposure as it concluded that exposure to boron between birth and puberty may result in adverse cellular effects that would "affect testicular function."

- EPA's *Summary Document from the Health Advisory for Boron and Compounds* provides an important warning regarding infants' exposure to boron in drinking water that is not included in either EPA's drinking water advisory for boron or its regulatory determination support document discussed above. Specifically, the summary document states that water containing boron "at levels above the HA [health advisory]" should not be used to prepare food or formula for infants. EPA does not identify which of the exposure duration health advisories it is referring to in this warning.

In addition, EPA's regulatory determination documents lack transparency and clarity regarding how EPA determined its health reference levels were protective of children. In addressing seven of the contaminants in its 2003 regulatory determination notice for which EPA identified children as a sensitive subpopulation, EPA did not explain the potential effect of not developing separate health reference levels for children (or not making adjustments to its health assessments to reflect increased sensitivity) on its ability to ensure that the health reference levels used in the regulatory determinations were protective of children. Instead, EPA stated that the agency had not yet determined a protocol for making a regulatory determination for a chemical for which body weight and drinking water intake of infants or a particular childhood age group would be the basis of a regulatory action. As discussed earlier, health assessments based on adult weight and drinking water intake may not fully account for the risks to children of exposure to drinking water because they consume more water per unit of body weight and may have other susceptibilities, as well. Regarding its 2008 notice that included four contaminants for which children were identified as a sensitive subpopulation, Office of Water officials told us they would have developed separate assessments for children if they had determined children were "particularly sensitive" to the adverse health effects of contaminants being considered for regulation. However, EPA did not explain in its regulatory determination notices or support documents the basis for its determinations that children were not particularly sensitive to the adverse health effects of the contaminants considered for regulation—even for those contaminants, such as manganese and boron, for which EPA had determined children were a sensitive subpopulation. EPA also did not explain how the sensitivity of children can be evaluated in the absence of a separate assessment based on the weight and drinking water intake of children.

Also, EPA's regulatory determination notices lack transparency and clarity regarding the limitations of new or updated health advisories the agency issued in conjunction with 9 decisions to not regulate. According to EPA, the advisories are to provide, for example, "guidance to communities that might be exposed to elevated concentrations." However, the regulatory determination notices do not acknowledge that when EPA determines regulation is not warranted but a health advisory is needed, it will generally be up to states, localities, and consumers to determine whether such contaminant levels are found in public water systems in their jurisdiction. Importantly, because public water systems are not typically required to test for the presence of unregulated contaminants, information on the levels of the contaminants in individual public water systems may be outdated or unavailable. While some states—such as California and Massachusetts—can promulgate their own drinking water regulations, others are statutorily prohibited from, or otherwise constrained in, enacting more stringent regulations than EPA has promulgated or promulgating their own drinking water regulations for contaminants that EPA does not regulate. In addition, individuals may have to have their water tested by a laboratory to determine how much of these unregulated contaminants are present in their drinking water to heed, for example, EPA's warning in some cases to not use drinking water with contaminants in excess of certain levels to prepare infant food or formula. Moreover, EPA releases its drinking water advisories by posting them on its Web site and does not issue public notification of them, such as a press release, which potentially limits awareness of the health advisories.

Our report provides information on the following limitations that also reduce the credibility of EPA's completed regulatory determinations: (1) EPA's explanations of the occurrence data EPA relied on to assess known and likely occurrence of contaminants in drinking water lack transparency, clarity, and consistency; (2) EPA's regulatory determinations lack clarity regarding its reliance on outdated and limited occurrence data to support some determinations; (3) the regulatory determination documents lack transparency and clarity regarding EPA's reliance on minimum reporting levels greater than its health reference levels; and (4) EPA lacked consistency and clarity in making determinations when IRIS assessments were either in process or needed to be updated.

The Process and Analyses EPA Relied on to Support Its Preliminary Determination on Perchlorate Were Atypical, Lacked Transparency, and Limited the Agency's Independence in Developing and Communicating Its Scientific Findings

As detailed in our report, we found the following concerning the process and scientific analyses EPA used to develop its 2008 preliminary determination to not regulate perchlorate.

EPA Used a Less Inclusive, Less Transparent, and More Directive Process in Developing Its Preliminary Regulatory Determination on Perchlorate Than Its Usual Process

In contrast to EPA's usual regulatory determination process, which is managed by a work group of professional staff with relevant expertise from across the agency, EPA officials decided that the agency's continuing deliberations on perchlorate would be managed by a less inclusive, small group of high-level officials, such as the Deputy Administrator and several Assistant Administrators. Notably, EPA did not include the Office of Children's Health Protection in its small group despite EPA's and the National Academies' conclusion that iodide uptake inhibition from perchlorate exposure had been identified as a concern in connection with increasing the risk of neurodevelopmental impairment in fetuses of pregnant women with iodine deficiency and to developmental delays and decreased learning capability in infants and children. This group of high-level officials managed the regulatory determination process for perchlorate both within EPA and externally with the

Perchlorate Interagency Working Group,[9] whose work was coordinated by the Council on Environmental Quality.[10] According to an EPA briefing document, the Perchlorate Interagency Working Group was established in 2002 "to identify and help resolve perchlorate science and science policy issues."

In contrast to the usual process EPA used for its regulatory determinations, in which EPA staff with relevant expertise develop and submit options to the Assistant Administrator for the Office of Water for review and selection, the Assistant Administrator directed the Office of Water staff in developing the preliminary determination for perchlorate to draft a preliminary determination that reflected the agency's decision to not regulate perchlorate and to support it with a detailed and specific rationale that EPA and other members of the Perchlorate Interagency Working Group had agreed to, under the leadership and coordination of the Council on Environmental Quality.[11] EPA Office of Water officials told us that they believed this agreement—which is not part of the record for the preliminary regulatory determination—was developed by senior officials from the Council on Environmental Quality, the Department of Health and Human Services (HHS), EPA, the Office of Management and Budget (OMB), and the U.S. Department of Agriculture. The agreement focused on how EPA should address the key science issues concerning perchlorate in its preliminary regulatory determination and specified (1) a health reference level of 15 parts per billion of perchlorate in drinking water and (2) the rationale for EPA to support the conclusion that this health reference level would be protective of pregnant women and their fetuses as well as of infants and children.

[9]The Perchlorate Interagency Working Group includes officials from the Office of Management and Budget (OMB) and the Office of Science and Technology Policy, both part of the Executive Office of the President; Department of Defense; National Aeronautics and Space Administration; Department of Energy; the Department of Health and Human Services' Food and Drug Administration and the Agency for Toxic Substances and Disease Registry; Department of Agriculture; and Department of the Interior.

[10]The Council on Environmental Quality, which is part of the Executive Office of the President, coordinates federal environmental efforts in the development of environmental policies and initiatives.

[11]According to an EPA official, the agreement was documented in an unattributed two-page white paper and faxed to EPA from the Council on Environmental Quality in early August 2008; EPA made some editorial changes to the document but did not alter the substance of the agreement.

EPA Established a Reference Dose for Perchlorate but Subjected It to a More Limited Review Than the Agency's Standard IRIS Assessment Review Process

In developing an IRIS assessment of perchlorate, EPA established a reference dose[12] on the basis of the National Academies' recommendations, but subjected it to a more limited review than the agency's standard IRIS assessment review process.[13] EPA's 2002 draft IRIS assessment of perchlorate—from which EPA derived a drinking water equivalent level of 1 part per billion[14]—drew significant attention—including from such federal agencies as the Department of Defense, the Department of Energy, and the National Aeronautics and Space Administration, because of the implications such a level could have on their operations if EPA were to develop a drinking water regulation for perchlorate. According to a senior EPA official, the controversy that arose over the draft IRIS assessment of perchlorate "was like nothing I had ever seen or have seen since." As a result of the divergent views between EPA and the other federal agencies, the Administrator of OMB's Office of Information and Regulatory Affairs urged the four interested agencies to convene a National Academies panel to review the draft IRIS assessment. Convened in October 2003, the panel conducted this review and issued its report in January 2005.[15]

The National Academies 2005 perchlorate report made several key recommendations to EPA on the basis of a different study from those on which EPA had based its 2002 draft IRIS assessment on perchlorate. The National Academies' recommended reference dose was more than 20 times higher than the one proposed in EPA's draft IRIS assessment. EPA's final internal review of the revised IRIS assessment for perchlorate—termed a consensus review—differed from the agency's

[12]A reference dose is an estimate of the total daily oral exposure to a contaminant—for example, from food and water—that is not likely to cause "appreciable risk of deleterious effects during a lifetime." A reference dose is a key component of the calculation EPA uses to derive a health reference level for drinking water contaminants.

[13]The National Academies consists of four private, nonprofit organizations that advise the federal government on scientific and technical matters: the National Academy of Sciences, National Academy of Engineering, Institute of Medicine, and National Research Council.

[14]A drinking water equivalent level represents the estimated exposure to a contaminant that is assumed to be protective for noncarcinogenic health effects during a lifetime of exposure. EPA calculated this drinking water equivalent level using the reference dose that EPA proposed in its 2002 draft IRIS assessment and the agency's default assumptions for adult weight and daily drinking water intake.

[15]National Academies, *Health Implications of Perchlorate Ingestion* (Washington, D.C., 2005).

usual consensus review process. For example, the scope of the internal review was limited in that the IRIS program did not seek input from consensus reviewers on the *scientific basis* for the assessment as it typically does; rather, it sought input only on the extent to which the science in the IRIS Summary was not inconsistent with the major conclusions of the National Academies' report. At least two EPA offices essentially opted out of the consensus review process because of this limitation, which was a significant departure from the usual IRIS consensus practice.

EPA Relied on an Estimate of the Relative Exposure to Perchlorate from Drinking Water and Food That It Derived from a Novel Analysis and Used a Nontraditional Method to Calculate the Relative Source Contribution

In developing its regulatory determination on perchlorate, EPA conducted a novel analysis to develop estimates of exposure to perchlorate for various subpopulations, which the agency subsequently used to calculate the relative source contribution—the allocated exposure to perchlorate from drinking water alone.[16] Independent scientists who reviewed EPA's analysis noted that it had several limitations—in particular, uncertainties specific to the exposure estimate for pregnant women. Nonetheless, EPA relied on the exposure estimate for pregnant women to calculate the relative source contribution, stating that the National Academies had identified pregnant women and their fetuses as the most sensitive subpopulation.[17]

In calculating the relative source contribution, EPA used a nontraditional method—called the subtraction method[18]—that was less conservative than the approach it had used for its other completed regulatory

[16]The relative source contribution is an allocation of the estimated oral exposure to the contaminant from drinking water alone; it has a significant impact on the health reference level that the agency derives for contaminants with noncancer adverse health effects.

[17]The 2005 National Academies report on perchlorate contained varying characterizations of sensitive subpopulations, sometimes referring to pregnant women and their fetuses alone as *the most sensitive subpopulation* and other times including infants in this designation. In addition, the National Academies identified developing children as a sensitive population and people with compromised thyroid function and people who are iodide-deficient as potentially sensitive populations.

[18]The subtraction method allocates the entire reference dose to the known sources of exposure by subtracting the known nontarget sources of exposure and allocating the remainder of the reference dose to the target—in this case, drinking water—even in cases where the total estimated exposure is less than the reference dose. This method has the effect of removing any cushion between the existing exposure levels and the reference dose.

determinations. While EPA identified some of the limitations of the exposure analysis in its preliminary regulatory determination notice for perchlorate, it did not discuss the effects of the limitations on EPA's exposure analysis. Although the agency's guidance for calculating the relative source contribution cautions against using the subtraction method in the absence of adequate data representative of at-risk populations—and EPA lacked data to estimate exposure to perchlorate for certain populations—the agency did not explain that the method it used to calculate the relative source contribution for perchlorate was the subtraction method or its reasoning for selecting this method.

According to Key EPA Scientists, the Agency Mischaracterized Important Scientific Findings That Emerged from Its Novel Analysis of the Sensitivity of Various Age Groups to Perchlorate in Drinking Water

In early 2008, EPA used a physiologically based pharmacokinetic (PBPK) model[19] to (1) evaluate the relative sensitivity of sensitive subpopulations to the health reference level the agency had developed based on pregnant women and their fetuses and (2) address concerns that some sensitive subpopulations, such as infants, exposed at the health reference level may receive concentrations of perchlorate above the reference dose. For its preliminary regulatory determination, the agency used the model in a novel way and, according to some key EPA scientists, mischaracterized the findings of the modeling analyses by selecting and presenting information in such a way as to support the agreed-upon conclusion that a health reference level of 15 parts per billion was protective of *all* sensitive subpopulations, including infants.

While EPA's Office of Research and Development conducted numerous sensitivity analyses with the PBPK model, EPA presented the results of a PBPK analysis in its October 2008 preliminary regulatory determination for perchlorate to support its conclusion that a health reference level of 15 parts per billion was protective of all sensitive subpopulations, including infants, and stated that using the model in this way could reduce some of the uncertainty regarding the sensitivities of subpopulations other than pregnant women. However, Office of Research and Development officials disagreed with the way EPA presented the information in its preliminary regulatory determination notice, saying the agency did not sufficiently explain the uncertainties and limitations of the analysis, presenting the information more conclusively than was appropriate.

[19]PBPK models are complex and involve numerous underlying assumptions that are imbedded in mathematical representations of the processes associated with how a contaminant behaves within, and is eliminated from, the body.

Further, the table EPA published in the preliminary regulatory determination notice presenting the results of the PBPK analysis included data that may not be consistent with EPA's conclusion that a health reference level of 15 parts per billion was protective of all subpopulations. That is, the table provided sufficient data for informed readers of the preliminary determination to calculate that infants and young children could be exposed to doses of perchlorate at levels as high as 5.5 times greater than the reference dose, supporting the concern that infants and young children may, in fact, be more vulnerable to perchlorate exposure. While EPA's regulatory determination notice stated that the modeled exposure exceeds the reference dose for some subpopulations, the agency was not explicit about the extent to which the reference dose is exceeded—as calculated above—and did not explain the implications of this result on its conclusion that the health reference level of 15 parts per billion is protective of all subpopulations. In providing comments on the draft notice to the Office of Water, an Office of Research and Development scientist noted that the agency's failure to present a comparison of the estimated daily exposure with the reference dose constituted a "serious omission," and characterized the infants' estimated exposure as "substantially higher" than the reference dose.

EPA's limited presentation of the PBPK analyses conducted by the Office of Research and Development in its preliminary regulatory determination notice validated the concern expressed at the time by Office of Research and Development scientists who conducted the analyses: that individual analyses could be used out of context in a way that could be misleading. Specifically, an Office of Research and Development official stated in September 2008 that while his office and the Office of Water had developed careful and sophisticated PBPK analyses to support the agency's preliminary regulatory determination, "the use of these science results in [the] draft regulatory determination is seriously flawed and misleading." As a result, Office of Research and Development officials and scientists that conducted the analyses concluded that the PBPK analysis done by the office did not support the draft preliminary regulatory determination's suggested health reference level of 15 parts per billion as being health protective for all sensitive subpopulations of concern to EPA.

EPA's Independence in Developing and Communicating Its Scientific Findings Was Limited by Its Acceptance of External Input on the Preliminary Determination Notice

Compounding scientists' concerns about the mischaracterization and lack of transparency regarding relevant scientific analyses, key language in EPA's preliminary regulatory determination notice appears to have been drafted by OMB rather than EPA. In working to finalize the preliminary regulatory determination notice, EPA's Office of Water worked with OMB, whose clearance of the notice was required per EPA's policy implementing Executive Order 12866 before the Office of Water could provide it to the EPA Administrator for review, approval, and publication in the *Federal Register*.[20] According to the Office of Water, in four iterations of review, OMB sent EPA a substantial number of comments on the notice; in response, EPA "clarified its description of the supporting analysis and strengthened the rationale for the determination." The following example highlights OMB's role in reviewing and approving the specific wording of EPA's scientific analyses regarding perchlorate exposure in infants and children:

> Text EPA provided to OMB: "Because infants and children eat and drink more on a per body weight basis than adults, eating a normal diet and drinking water with 15 [micrograms per liter] of perchlorate *is likely to* result in exposure that is greater than the reference dose in these groups."
>
> Revised text provided to EPA by OMB: "Because infants and children eat and drink more on a per body weight basis than adults, eating a normal diet and drinking water with 15 [micrograms per liter] of perchlorate *may result in* exposure that is greater than the reference dose in these groups."

By changing three words, OMB downplayed EPA's characterization of the health risks of perchlorate exposure. Importantly, the EPA scientist who wrote the text provided to OMB noted to EPA reviewers—before it was sent to OMB in August 2008—that the PBPK model actually showed exposures at levels "much higher" than the reference dose, but also said that he believed describing the exposure scenario as "likely" was the strongest characterization that might be retained through OMB review. In addition, in September 2008, during its review of the draft preliminary determination notice and before clearing it for publication, OMB reminded

[20]The objectives of this executive order are to enhance planning and coordination with respect to both new and existing regulations; to reaffirm the primacy of federal agencies in the regulatory decision-making process; to restore the integrity and legitimacy of regulatory review and oversight; and to make the process more accessible and open to the public.

EPA that it expected the notice to "state a clear conclusion that the HRL [health reference level] is protective of all subpopulations, as agreed to in the August framework"—and accordingly, this conclusion appeared in the agency's October 2008 preliminary determination notice.[21]

EPA's Response to Our Recommendations Does Not Appear to Acknowledge the Scope and Significance of the Implementation Limitations We Identified

We provided a draft of this report to the Administrator of EPA for review and comment. In commenting on the draft report, EPA agreed with 2 of the 17 recommendations we made to improve its implementation of the Safe Drinking Water Act. Specifically, EPA agreed with our recommendations regarding its drinking water health advisories, stating that it would evaluate their utility and determine whether and how to revise the advisories to better serve states, localities, public water systems, and the public. However, EPA did not agree to implement the remaining 15 recommendations we made, including an overarching recommendation that EPA develop policies or guidance that clearly articulate the agency's interpretation of the Safe Drinking Water Act's broad statutory criteria—as well as eight additional recommendations identifying specific components of this guidance and calling for review of the draft guidance by one of EPA's independent advisory committees and the establishment of an internal review mechanism to help ensure the determinations are consistent with the guidance. Another key recommendation with which EPA disagreed was for EPA to include in the public record OMB's and other federal agencies' comments on and revisions to regulatory determination notices and support documents to improve transparency and help EPA ensure that it maintains the fairness and openness of its operations.

We made these recommendations to support the development of regulatory determinations that are transparent, clear, and consistent and that follow applicable agency policy. However, EPA said it believed that establishing policies or guidance for regulatory determinations was not "practicable" because of the many combinations of health effects factors and potential ranges of frequencies and levels of contaminants measured in drinking water. We do not believe that the existence of variables or complexities is a basis for not developing guidance for EPA staff to implement the statutory requirements for regulatory determinations. In

[21]According to an EPA official, "August framework" refers to the agreement that was faxed to EPA from the Council on Environmental Quality that included this conclusion as a key component of the rationale EPA and other federal agencies agreed to in August 2008.

fact, the complexities cited would argue for, rather than against, the need to develop guidance for staff on applying the criteria. EPA also did not agree with these recommendations on the basis that policies or guidance could "inhibit its ability to continually improve its actions." This perspective suggests that guidance *per se* lacks flexibility. We do not agree that guidance and flexibility are incompatible or that developing guidance would inhibit EPA's ability to improve its actions. Rather, flexibility can and should be incorporated into guidance by establishing parameters or options for areas in which flexibility is deemed appropriate.

Moreover, consistency and accountability are lacking in this important program because EPA has not developed guidance on the application of the broad statutory criteria, which are susceptible to varying interpretations. In its comments, EPA highlighted that, under these criteria, ultimately it is the Administrator's judgment as to whether regulation of a contaminant in drinking water presents a meaningful opportunity for health risk reduction, after considering the information presented by agency staff. As stated in our report, the statutory criteria are so broadly stated that they could potentially be interpreted so as to lead to regulating all the contaminants on the candidate list, some of them, or none of them. It is precisely for these reasons that we believe it is essential for the staff to have sufficient guidance on applying the broad criteria consistently and transparently so that the Administrator's judgment can be based on sound and consistent information. Without such guidance, the basis for EPA's determinations and the quality of the documentation the staff use to support them can fluctuate over time as a result of, among other reasons, changes in agency leadership and staff. In addition, regarding our recommendation that EPA provide in the public record OMB's and other federal agencies' comments on and revisions to regulatory determination documents, EPA's position is that unless required by law, it is not a good policy because, among other things, the documents may be confusing to the public and undermine the ultimate policy choice. We disagree and believe that to improve transparency of these determinations, which are by law committed to the Administrator's judgment, EPA should consistently make these documents available in the public record, regardless of whether there is a specific legal requirement for disclosure.

In large measure, EPA's response to our recommendations essentially endorses conducting business as usual; a response that does not seem to acknowledge the scope and significance of the implementation limitations we identified. We are concerned that EPA's lack of responsiveness to our recommendations may reflect a misplaced reliance

on the Office of Water to independently improve the management of this important program in the absence of (1) regulations, policies, or guidance that we believe are needed to provide a framework for current and future staff to apply in identifying and evaluating contaminants for regulation; (2) the identification of clear and specific actions needed to address our recommendations; and (3) an internal review mechanism to ensure identified actions are implemented effectively. We believe that EPA needs to adopt all of the recommendations in our report to better assure the public of safe drinking water.

Madam Chairman Boxer, Ranking Member Inhofe, and Members of the Committee, this concludes our prepared statement. I would be happy to respond to any questions that you or other Members of the Committee may have.

Contacts and Acknowledgements

For further information about this testimony, please contact me at (202) 512-3841. Christine Fishkin, Jamie Meuwissen, Elizabeth Beardsley, Kiki Theodoropoulos, and Michael Derr also made key contributions to this statement.

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November 3, 2011

The Honorable Barbara Boxer
Chairman
The Honorable James M. Inhofe
Ranking Member
Committee on Environment
and Public Works
United States Senate

Attention: Jonathan Aronchick

We appreciate having had the opportunity to appear before the Senate Committee on Environment and Public Works on July 12, 2011, to speak about GAO's work on EPA's implementation of the Safe Drinking Water Act provisions related to unregulated drinking water contaminants.

Enclosed are GAO's responses to the questions you submitted for the hearing record related to our testimony, *Safe Drinking Water Act: Improvements in Implementation Are Needed to Better Assure the Public of Safe Drinking Water,* GAO-11-803T, July 12, 2011. If you or your staff have any questions, please contact me at (202) 512-3841 or TrimbleD@gao.gov.

David C. Trimble
Director, Natural Resources
and Environment

Enclosure

cc: Grant Cope
Elizabeth Fox

Enclosure

GAO Response to Questions for the Record

Senate Environment and Public Works Committee Hearing

"Oversight Hearing on the Environmental Protection Agency's Implementation of the Safe Drinking Water Act's Unregulated Drinking Water Contaminants Program"

July 12, 2011

Response to Questions Submitted by Senator Barbara Boxer

1. The GAO found that EPA lacks "criteria and a process for identifying those [drinking water] contaminants on its candidate list that pose the greatest public health concern..." Could you please explain the practical importance of this type of criteria for ensuring transparency and accountability in EPA's decisions to regulate or not regulate drinking water contaminants?

Establishing criteria and a process for identifying contaminants on the candidate lists that present the greatest public health concern is important if EPA is to target its limited resources on obtaining the data it needs to support regulatory determinations on priority contaminants. The Safe Drinking Water Act requires EPA to select contaminants for consideration for regulatory determinations that present the greatest public health concern. As we reported,[1] data availability—not consideration of greatest public health concern—has been the primary driver of EPA's selection of contaminants for regulatory determinations. Moreover, EPA has reported that it lacked sufficient occurrence or health effects data, or both, for making regulatory determinations for at least 100 of the contaminants on the current (2009) contaminant candidate list, which includes 17 contaminants that have been on all three candidate lists. In light of increased fiscal constraints and programmatic demands, it is increasingly important for EPA to establish criteria and a process so that it can focus its resources on those contaminants that may pose the greatest public health concern. The increased transparency provided by criteria and a defined process would support EPA's compliance with the statutory requirement for prioritization and provide a basis for EPA's accountability for its decisions, with the overarching goal of better assuring the public of safe drinking water.

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[1]GAO, *Safe Drinking Water Act: EPA Should Improve Implementation of Requirements on Whether to Regulate Additional Contaminants*, GAO-11-254 (Washington, D.C.: May 27, 2011).

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2. The GAO recommended that EPA develop and allow the public to review guidance that describes the Agency's interpretation of the Safe Drinking Water Act's criteria for making regulatory determinations. GAO recommended that such guidance specify thresholds that must be met to support a regulatory finding; factors for determining when monitoring and health effects data are adequate to support such a finding; and the drafting of comprehensive, consistent and understandable documents. Given the GAO's review of EPA's current practices, how significant are the changes contemplated by these recommendations?

The changes are potentially significant in that they seek to improve the consistency and accountability of this important program. Accountability and consistency has been lacking in the program, in part, because EPA has not developed guidance on the application of the broad statutory criteria, which are susceptible to varying interpretations. As we reported, the statutory criteria are so broadly stated that they could potentially be interpreted so as to lead to regulating all the contaminants on the candidate list, some of them, or none of them. It is precisely for these reasons that we believe it is essential for EPA staff to have sufficient guidance on applying the broad criteria consistently and transparently so that the Administrator's judgment can be based on sound and consistent information. Without clarifying guidance, EPA's regulatory determinations lack transparency, and EPA is at risk of making inconsistent determinations, undermining the program's credibility and the agency's ability to assure the public of safe drinking water. Based on the findings in our report, we recommended that EPA develop and make available to the public policies or guidance that clearly articulates the agency's interpretation of the Safe Drinking Water Act's broad statutory criteria for making regulatory determinations and that the guidance should, among other things: (1) specify any thresholds or parameters that the agency requires to be met to support a positive finding; (2) include factors for determining when the occurrence and health effects data the agency identifies are adequate to support a regulatory determination; and (3) establish a process to ensure that the presentation of health effects and occurrence information in regulatory determination notices and support documents is comprehensive, consistent, informative, and understandable. We believe that EPA needs to adopt all of the recommendations in our report to better assure the public of safe drinking water, including and especially, developing and implementing regulations, policies, or guidance to provide a framework for current and future staff to apply in identifying and evaluating contaminants for regulation.

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3. The GAO found that the Office of Water "has not developed guidance for when and how to analyze the effects of drinking water contaminants on children..." The GAO also recommended that EPA create "approaches...to evaluate [contaminants] health effects on sensitive subpopulations, including such groups as infants and children..." If EPA develops this type of guidance, could it help to ensure that the Agency considered children's health issues in a more consistent and transparent fashion that it has in the past? Are there other potential benefits?

Developing and implementing the guidance we recommended could help ensure that EPA considers children's health issues in a more consistent and transparent way than it has in the past. Moreover, if implemented effectively, the guidance could help ensure that EPA's regulatory determinations fully consider the subpopulations that are the most sensitive to adverse health effects from drinking water contaminants, rather than just the average adult. As we reported, EPA Office of Water's lack of guidance for when and how to analyze the effects of drinking water contaminants on children in its regulatory determinations hampers EPA's ability to consistently apply and document (1) the Safe Drinking Water Act requirement to consider sensitive subpopulations when selecting contaminants for regulatory determination and (2) EPA's children's health policy, which specifies how health risks to infants and children should be analyzed when the agency is developing a policy, regulatory action, or decision. For example, in the 2008 regulatory determinations, the Office of Water did not implement a specific approach to considering children's health. Further, it did not explain how or whether the agency determined that a separate assessment for children or an adjustment to the health reference level was not warranted.

4. The GAO found that in 2008, EPA's "Assistant Administrator [for Water] directed the staff to develop a determination to not regulate perchlorate and to support a specific exposure level as protective of all populations...[as] outlined in an agreement between high-level officials at EPA and other federal agencies..." GAO also found that "key language" in the determination "notice appears to have been drafted by OMB rather than EPA." In your opinion, will requiring transparency with these types of activities help to ensure accountability in government decision-making and also help to ensure better use of science in making regulatory determinations?

By implementing our recommendation to include in the public record OMB's and other federal agencies' comments on and revisions to regulatory determination notices and support documents, EPA could help ensure accountability in government-decision making and could help ensure better use of science in EPA's regulatory determinations. For example, as we reported, by changing three words in EPA's preliminary regulatory determination for perchlorate,

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OMB downplayed EPA's characterization of the health risks of perchlorate exposure, and overall, the changes EPA made in response to OMB's comments presented EPA's conclusions with greater certainty than key EPA scientists stated they were comfortable supporting. Making public OMB's and other federal agencies' comments on and revisions to regulatory determination notices and support documents is key to enhancing the public's understanding of the degree to which EPA is maintaining its independence in developing and communicating the results of its scientific analyses.

5. The GAO found that 9 of EPA's 20 determinations not to regulate a contaminant involved data from monitoring activities that could not detect these contaminants at levels that posed a health concern. The GAO recommended that EPA develop a process to "reconsider whether to regulate a contaminant" that it has determined not to regulate, and to "consider whether the agency needs to reevaluate any of its past determinations to not regulate." Could you please describe the importance of EPA developing this type of guidance for protecting public health?

It is important that EPA develop guidance establishing a process for when and how it will re-evaluate contaminants for which new or revised scientific information becomes available in order to (1) provide transparency and clarity regarding the circumstances under which EPA would undertake a re-evaluation of a completed regulatory determination and (2) help ensure consistency in the re-evaluations. Under the 1996 amendments of the Safe Drinking Water Act, EPA is to select for consideration those unregulated contaminants that present the greatest public health concern, evaluate their occurrence and the potential health risks associated with them, and decide whether a regulation is needed for at least five contaminants every 5 years. While EPA officials told us that EPA could reconsider a determination to not regulate a contaminant on the basis of new scientific data, such as a new IRIS health assessment, the agency has not developed any guidance on the circumstances that would trigger such a re-evaluation. For example, in the case of 1,1,2,2-tetrachloroethane, EPA made a determination to not regulate the contaminant in 2008.[2] In 2010, EPA's IRIS program finalized an updated health assessment, reclassifying 1,1,2,2-tetrachloroethane from a "possible" to a "likely" human carcinogen. However, the agency has not announced whether it will reconsider its determination for this contaminant. In addition, in 2003, EPA made a determination to not regulate dieldrin on the basis that its occurrence in drinking water at frequencies or concentrations significant for

[2]Prior to the 1980s, 1,1,2,2-tetrachloroethane (a volatile organic compound, or VOC) was used in the production of other chemicals, such as trichloroethylene (TCE) and tetrachloroethylene (PCE). It was also used as a metal degreaser and a component of paint removers, varnishes and lacquers, and photographic films. While no longer generated as an end product, it is still produced as a byproduct in the manufacturing of other synthetic chemicals.

public health concern is low, according to EPA data.[3] As we reported, however, using more sensitive tests than EPA used, the U.S. Geological Survey (USGS) conducted a study from 1993 to 2007 in which it detected dieldrin in 3.1 percent of public well samples.[4] Importantly, essentially all of the detections were at levels that exceeded EPA's health benchmark and would not have been found if USGS had used the reporting levels on which EPA based its regulatory determination. As we reported, the decisions to not regulate these contaminants were made in the absence of policies or guidance on applying the broad statutory criteria in the Safe Drinking Water Act. We continue to believe it is important for EPA to develop the guidance we recommended—and consider whether the agency needs to re-evaluate any of its past determinations to not regulate in order to better assure the public of safe drinking water.

6. The GAO found that 17 contaminants have been on all three of EPA's lists of priority drinking water contaminants since 1998, including RDX or "Royal Demolition Explosive," which EPA has classified as a possible human carcinogen. The GAO also raised concerns with a potential lack of information that EPA has on some of these contaminants. What types of issues do these 17 contaminants raise concerning EPA's implementation of the unregulated contaminant program?

The primary concern for which these 17 contaminants serve as examples is that a lack of coordinated occurrence and health effects data continues to limit EPA's ability to make timely regulatory determinations on contaminants of concern to EPA. EPA has identified these 17 contaminants as candidates for drinking water regulation since 1998 and yet has been unable to make determinations on them because of insufficient occurrence or health effects data. While data on exposure and health effects from similar time frames are generally needed to develop credible regulatory determinations and drinking water regulations, EPA's current processes for conducting Integrated Risk Information System (IRIS) assessments and selecting contaminants for its unregulated contaminants testing program are not producing such data. EPA is collecting nationally representative occurrence data on some contaminants to identify exposure at levels of public health concern but is not at the same time actively pursuing IRIS assessments or other credible sources for health effects information.

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[3]Dieldrin is an insecticide that was banned by EPA in 1974 for most uses, except for the control of termites, and banned for all uses in 1987 because of concern about environmental damage, harm to human health, and its ability to persist in the environment for decades.

[4]USGS, *Quality of Source Water from Public-Supply Wells in the United States*, 1993-2007 (Reston, Va., May 2010).

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Response to Questions Submitted by Senator James M. Inhofe

1. Why did GAO not take into account the EPA Inspector General's review of perchlorate in both its 2010 report and this report?

The EPA Inspector General's science review of perchlorate—which included an alternative health risk assessment developed by the Inspector General—was published in 2010, 2 years after EPA's preliminary regulatory determination on perchlorate and thus was not relevant to the objectives of our 2011 report.[5] The objectives of the 2011 report were to (1) evaluate the extent to which EPA's implementation of the 1996 amendments' requirement for determining whether to regulate potentially harmful contaminants has helped assure the public of safe drinking water and (2) review the process and scientific analyses EPA used to develop its 2008 preliminary regulatory determination on perchlorate. We do cite the EPA Inspector General's report on perchlorate in our 2010 report.[6] However, we did not include detailed information on the conclusions of the EPA Inspector General's report because the issues included in it were beyond the scope of our report. Our 2010 report[7] examined (1) what is known about the extent to which perchlorate occurs in the nation's water and food supply and its likely sources; (2) what actions DOD, NASA, and DOE have taken to respond to or lessen perchlorate releases; and (3) what actions states, such as California and Massachusetts, have taken to regulate perchlorate.

2. You highlighted a lack of transparency as a major issue in the Unregulated Drinking Water Contaminants Program. What benefits would the Agency and the Public obtain from consistent guidance on how EPA is interpreting and using its statutory authority?

By developing guidance that specifies how EPA is interpreting and using its statutory authority, the agency will establish a framework for more credible decision making and increased accountability. For example, establishing criteria or parameters related to what level and frequency of contaminant occurrence may warrant regulation and its definitions of what characterizes a contaminant of "greatest public health concern" and "meaningful opportunity" for public health risk reduction will provide: (1) a framework to guide EPA program staff through the data gathering and analytical stages of the regulatory determinations and (2) benchmarks that

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[5]GAO-11-254.

[6]EPA, *Office of Inspector General Scientific Analysis of Perchlorate*, Report No. 10-P-0101 (Washington, D.C., April 2010).

[7]GAO, *Perchlorate: Occurrence Is Widespread but at Varying Levels; Federal Agencies Have Taken Some Actions to Respond to and Lessen Releases*, GAO-10-769 (Washington, D.C.: Aug. 12, 2010).

EPA can use to support and defend its science-based regulatory determinations to protect public health. The increased transparency that would result from EPA developing the guidance we recommended would support the agency's implementation of the statutory requirements and provide a basis on which EPA can be held accountable for its decisions, with the overarching purpose of better assuring the public of safe drinking water.

3. Do deficiencies in IRIS affect EPA's ability to make regulatory decisions in the Drinking Water program?

IRIS assessments provide certain scientific data on health effects that EPA needs to develop drinking water health reference levels—the benchmark the agency uses to evaluate whether contaminants occur in public drinking water at levels of public health concern. The lack of productivity of EPA's IRIS program has impeded effective implementation of EPA's regulatory determinations. As we reported, EPA identified health effects information gaps for 44 of the 104 chemicals on its third candidate list published in 2009. However, the number of contaminants with health effects information gaps may be higher because there are another 24 contaminants on the candidate list for which EPA does not have an IRIS assessment or for which an IRIS assessment is either in progress or has not yet begun. As you know, because EPA had not developed sufficient chemical assessment information to limit public exposure to many chemicals that may pose substantial health risks, in January 2009 we added EPA's processes for assessing and controlling toxic chemicals to our list of areas at high risk for waste, fraud, abuse, and mismanagement or—as is pertinent to EPA's implementation of the IRIS program and the Toxic Substances Control Act—in need of enhanced oversight and transformation.[8]

[8]GAO, *High-Risk Series: An Update*, GAO-09-271 (Washington, D.C.: January 2009); and *High Risk Series: An Update*, GAO-11-278 (Washington, D.C.: February 2011).

Senator BOXER. Thank you very much for your hard work.

And now it is time to hear from the EPA, and Mr. Perciasepe, welcome.

STATEMENT OF HON. ROBERT PERCIASEPE, DEPUTY ADMINISTRATOR, ENVIRONMENTAL PROTECTION AGENCY

Mr. PERCIASEPE. Thank you. Thank you for inviting me today, Chairman Boxer and Ranking Member Inhofe and members of the Committee here today to discuss EPA's drinking water program.

Making sure that all Americans have water that is safe to drink is one of the fundamental elements of EPA's mission. I appreciate the Committee's and the Government Accountability Office's attention to the matter of the Safe Drinking Water Act. While EPA, under the leadership of Administrator Jackson, has made some key strides with the drinking water program, there is always room for improvement, and the GAO report makes some critical recommendations which we welcome.

Strong science and the law are the foundation of our decision-making at EPA. We agree with GAO that our approach to drinking water regulation must be transparent, consistent, clear and driven by the need to protect public health. In 1996, Congress updated the Safe Drinking Water Act to improve the way we decide when a contaminant needs to be regulated. The new process was designed to assure that future standards focus on public health protection and are based on the best available science.

Over the years, we have sought advice from a number of independent organizations on how to achieve this goal, including the National Academy of Sciences, the Science Advisory Board, and the National Drinking Water Advisory Council. Recommendations from these groups have contributed to a strong foundation for our program, and we continue to build on those past efforts. The draft GAO report makes it clear we have more work to do.

Specifically, GAO provides recommendations about developing policies to interpret the broad statutory criteria that the Safe Drinking Water Act outline for making regulatory determinations. It is crucial that these criteria are transparent and protect vulnerable populations, particularly children. To achieve these goals, I have asked the Office of Water to consult with an independent panel of scientists on the process EPA uses.

Specifically, they will consider how we evaluate contaminants against the criteria, how will we use the best available science to make a decision and how will we assess the greatest public health risk and how to consider vulnerable populations, especially children. We will make our regulatory determination process publicly available and we will review the process every 5 years as we conduct the regulatory determination cycle.

Currently, we are in our third cycle of evaluating unregulated contaminants. In implementing this process, we are applying the lessons learned from the previous iterations. Our recent actions are consistent with many of GAO's recommendations. To ensure that we are best protecting public health, GAO recommends that EPA develop criteria to identify contaminants that pose the greatest risk. In our most recent contaminant candidate list, EPA used a significantly improved process. We cast a wide net to identify pos-

sible contaminants. And from this universe of more than 7,000 candidates, we narrowed the list down to 116 that represent the greatest risk. The multi-step process was science-based and informed by the public and independent science experts. All of the supporting information is available to the public. From within this list, we have identified the contaminants with enough data to make a decision in this cycle, and we are focusing our decision on those with the greatest public health concern.

In order to have the occurrence data we need to make good decisions, GAO recommends that we improve our unregulated contaminants monitoring program. We agree with the GAO's recommendations . In March, we proposed our most recent list of contaminants for monitoring and we are taking full advantage of the statutory authority to test for 30 contaminants. We also proposed minimum reporting limits that are much lower than have been required in the past, making the data obtained more useful.

This Administration also recognizes that we need to change the paradigm for determining and developing drinking water regulations to protect public health. We are implementing the drinking water strategy announced by the Administrator last year. In particular, we have established a focus on regulating multiple contaminants as a single group. By doing so, we can achieve public health protection more expeditiously and in a way that is more cost-effective for water utilities to implement.

We are also focusing on fostering innovative drinking water technology, so that drinking water treatment is available and affordable. We are leveraging the authorities of multiple statutes to protect drinking water sources from contamination.

In closing, Madam Chairman, protecting the resource of clean, safe water is one of EPA's highest priorities. We are committed to continually improving our methods and I look forward to working with this Committee and the GAO in our efforts to best protect the American people.

Thank you.

[The prepared statement of Mr. Perciasepe follows:]

Testimony of
Robert Perciasepe, Deputy Administrator
U.S. Environmental Protection Agency

Before the
Committee on Environment and Public Works
United States Senate

July 12, 2011

Chairman Boxer, Ranking Member Inhofe, and Members of the Committee, thank you for inviting me to discuss how the EPA decides when to set new drinking water standards. The public relies on EPA to ensure the safety of the water they drink every day, and EPA takes this responsibility very seriously.

Strong science and the law are the foundation of our decision-making at EPA. Under the Safe Drinking Water Act (SDWA), EPA identifies priority contaminants that are known or anticipated to occur in public water systems and then evaluates whether new drinking water standards are warranted for these contaminants. EPA appreciates the Government Accountability Office's (GAO) attention to the important matter of setting drinking water regulations and we welcome GAO's input about how to do this most effectively. EPA has reviewed GAO's draft report so my testimony reflects my consideration of the recommendations in that version. GAO's draft recommendations address three key areas for EPA to improve implementation of requirements on whether to regulate additional contaminants:

1. Development of criteria to identify contaminants that pose the greatest health risk;

2. Improvement of EPA's unregulated contaminants testing program, and

3. Development of policies or guidance to interpret the broad statutory criteria.

EPA agrees with GAO that consistency, transparency and clarity are essential in assuring the safety of public drinking water and our credibility with the public. While we have made substantial progress in achieving this goal, we agree that there is room for improvement. We are committed to actions to ensure that the public has confidence that the EPA's decisions are protective of their health and based on a thorough consideration of the best available science and information.

EPA is in the third cycle of evaluating unregulated contaminants as required by the 1996 SDWA amendments. EPA has completed the third Contaminant Candidate List (CCL), proposed the third Unregulated Contaminant Monitoring Rule (UCMR3), is developing the third round of regulatory determinations, and also recently made the off-cycle determination to regulate perchlorate. We are continually learning from each iteration of this process and are currently applying lessons learned from previous determinations. We believe the improvements we have made go a long way towards addressing GAO's concerns.

Administrator Jackson also announced last year a new vision for better protecting drinking water including changing the paradigm of evaluating individual contaminants for regulation. Under the new drinking water strategy, EPA is committed to:

1. Considering regulation of groups of contaminants to better protect public health by streamlining decision-making and in a way that is likely more cost-effective for water systems to implement
2. Fostering development of new drinking water technologies

3. Leveraging other regulatory programs such as the Toxic Substances Control Act and the Federal Insecticide, Fungicide, and Rodenticide Act as appropriate to protect sources or drinking water

4. Working with states to share more complete drinking water monitoring data to support evaluation of drinking water protection nationwide and make information accessible to the public.

We are implementing this strategy as we conduct the ongoing cycle of regulatory determinations.

Identifying Contaminants of Concern

The first step of the evaluation is to identify the contaminants of greatest public health concern, which EPA does through the Contaminant Candidate List. In the most recent CCL, published in October 2009, EPA used the advice from the National Academy of Sciences and the National Drinking Water Advisory Council to develop and usea significantly improved, more transparent and reproducible multi-step process to ensure more effective identification of public health threats. We cast a wide net in identifying possible drinking water contaminants including those nominated by the public. From an initial universe of 7,500 contaminants, EPA evaluated available occurrence and potential health effects data for this universe and incorporated public input and expert review. Through this review, we selected from this universe a list of 116 priority contaminants that we found to be of the greatest public health concern based on both the severity of the health effect and the anticipated occurrence. EPA also improved transparency by making all data and criteria used to classify contaminants publicly available on the EPA's website.

GAO's report expresses concern that EPA's past decisions have been driven not by considering the greatest health concern but by considering available data. EPA agrees that we can improve our process to better focus on contaminants that may be of public health concern. The improved approach in the most recent CCL was a substantial step forward in achieving this by using a rigorous scientific process to better ensure that the contaminants on the list are the ones that should be of highest priority for public health protection. Because the new CCL selection process targeted candidates based on possible health effects or exposure rather than just on available data, the list includes emerging contaminants that are not currently well enough understood to discern whether regulation of drinking water could improve public health. EPA cannot make a credible decision driven by science without sufficient understanding of the potential for impacts to the health of the American people. Therefore, EPA has since narrowed the CCL down to a "short list" of 32 contaminants that have sufficient data to make a determination within the statutory timeline. This short list is being prioritized for regulatory decision making in this cycle based on the greatest public health concern. Those determinations will be announced by next summer for public comment. We believe this approach addresses GAO's concerns with previous cycles of our process.

Collect and Evaluate Information

For the remaining candidates, obtaining robust data and information regarding potential impacts is essential. For the current CCL, the evaluation of contaminants included a discussion of data gaps so that further information can be collected to support

future decisions. The CCL classifies the contaminants based on the need for health effects data or occurrence data or analytical methods.

To obtain occurrence data, EPA uses the Unregulated Contaminant Monitoring Rule (UCMR) and also looks to data collected by others such as the states and the U.S. Geological Survey. GAO had a number of recommendations to improve the UCMR process. We agree with the GAO recommendations regarding UCMR. They are consistent with the most recent UCMR proposal, published in March, in which EPA looked at health effects information to target the contaminants of greatest concern. We also proposed, as GAO has recommended, to use our full statutory authority to require testing for 30 contaminants and to conduct full assessment monitoring rather than more limited screening surveys. Additionally, the proposed UCMR generally requires much lower minimum reporting levels than have been required in the past, making the data obtained more useful in determining the likelihood of health impacts when contaminants are detected.

Good data about health effects are also essential, and EPA's Office of Water identifies priority contaminants and health assessment information needs and coordinates with the EPA's Office of Research and Development and external organizations. The Agency searches the available literature and participates in scientific meetings to identify evolving science that may support evaluation of health effects. EPA has also made substantial revisions to the Integrated Risk Information System (IRIS) process to provide assessments in a timely fashion to best support regulatory decisions. EPA has reduced the IRIS backlog and shortened the risk assessment development time while ensuring

rigorous peer review. Strengthening and streamlining the IRIS process is a continuing and ongoing priority for EPA.

Regulatory Determination

According to SDWA, EPA must make determinations for at least five contaminants from each CCL. SDWA defines three criteria to determine whether it is appropriate for EPA to regulate a potential drinking water contaminant:

- The contaminant may have an adverse effect on the health of persons;
- The contaminant is known to occur or there is a substantial likelihood the contaminant will occur in public water systems with a frequency and at levels of public health concern; and
- In the sole judgment of the Administrator, regulation of the contaminant presents a meaningful opportunity for health risk reductions for persons served by public water systems.

EPA determines whether an adverse health effect may occur, identifies what levels of exposure may result in public health concern and then evaluates how extensive potential exposure at those levels might be. Finally, the Administrator must decide whether regulatory action taken by EPA would serve to reduce public health risk in a meaningful way. A decision by the Administrator to regulate a contaminant is the beginning of the SDWA regulatory development process. EPA has extensive further requirements regarding analyses of health benefits, costs, and treatment technologies that must be conducted before a National Primary Drinking Water Regulation is proposed and made final.

In the first two cycles of regulatory determinations, EPA made negative determinations for 20 contaminants, in each case deciding that the occurrence of the contaminant was not at a frequency and level of public health concern to merit a new drinking water standard. This February, EPA made the Agency's first positive determination, when we announced that we will be developing a proposed drinking water standard for perchlorate by February 2013 at the latest.

Strong science must be the foundation of decisions regarding the criteria defined by SDWA, but science alone cannot fully address the criteria. As the GAO describes in their report, there are a large number of factors that can impact our understanding of what levels would be of concern and how likely those are to occur, such as the severity of health effects, the potency of the contaminant, the geographic distribution and levels of drinking water detections, or other possible sources of exposure. In its regulatory determinations, EPA has sought out and evaluated available information on these factors and based our determinations on our best understanding of the existing information.

Given the many possible combinations of factors and the constantly evolving science, it is essential that the bases for EPA's decisions be clearly presented so that the public can have confidence in our actions. For our regulatory determinations, our *Federal Register* notices and supporting documentation list the primary occurrence and health effects data, describe our evaluation of whether this information is sufficient, and explain our approach for deriving endpoints.

The concerns that GAO raises indicate that we have not always done this effectively enough. We will do a better job in the future. EPA will work to improve the

transparency of regulatory determination so that the public can better understand how EPA came to its conclusions and most effectively comment or review. EPA will make this information available when we publish our preliminary determination.

In response to the GAO recommendations, EPA will also consult with an independent panel of scientists on the regulatory determinations, specifically on the evaluation of the contaminants against the first and second criteria defined by SDWA, the use of best available science to develop the determination, and whether the determination focuses on the greatest public health risk. We will post the regulatory determination process publicly and review the process every five years as we conduct the regulatory determination cycle.

Regulating Contaminants as Groups

As I stated earlier, in parallel to these improvements to the standard regulatory determination process, EPA is changing the regulatory approach that has primarily addressed contaminants one at a time. In February, Administrator Jackson announced that we are developing a single regulation to include up to 16 volatile organic compounds that may cause cancer. Several of these contaminants are on the current CCL and others are currently regulated and need to be revised. By considering them as part of a group rather than through individual regulatory determinations, we can address the public health concerns from a larger portion of our priority list at one time, achieving greater health protection more expeditiously and in a way that is likely to be more cost-effective for utilities to implement.

In the current round of regulatory determinations, EPA is also evaluating whether to regulate nitrosamines (currently on the CCL) as a group. We have found these disinfection byproducts in a number of water systems and considering them as a group would allow us to take advantage of shared analytical methods and treatment or control processes, as well as making a greater impact on public health because nitrosamines sometimes co-occur, and because controlling nitrosamines also reduces exposure to related disinfection byproducts.

Conclusion

Clean and safe water is the foundation of healthy communities, healthy families, and a healthy economy. EPA is committed to continuing to improve our methods in using science and the law to best protect public health. I greatly appreciate the leadership of this Committee in helping to protect drinking water. We look forward to coordinating with this Committee as we work to achieve these important goals.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 12 2012

OFFICE OF CONGRESSIONAL AND
INTERGOVERNMENTAL RELATIONS

The Honorable Barbara Boxer
Chairman
Committee on Environment and Public Works
United States Senate
Washington, D.C. 20510-6175

Dear Chairman Boxer:

Thank you for the opportunity to respond to the questions for the record following the July 12, 2011 hearing on "The EPA's Implementation of the Safe Drinking Water Act's Unregulated Drinking Water Contaminants Program." The attached document has responses to the questions. I hope that this information is useful to you and the members of the committee.

If you have any further questions, please contact me or your staff may call Sven-Erik Kaiser in my office at (202) 566-2753.

Sincerely,

Arvin Ganesan
Associate Administrator

Attachment

Senate Committee on Environment and Public Works
"Oversight Hearing on the EPA's Implementation of the Safe Drinking Water Act's Unregulated Drinking Water Contaminants Program"
Questions for the Record
July 12, 2011

Senator Boxer

Boxer 1. The GAO found that "EPA does not have criteria for identifying contaminants of greatest public health concern ..." and recommended that EPA develop criteria to identify contaminants that pose the greatest public health risk."

Will EPA develop such criteria in order to determine which drinking water contaminants should be regulated and ensure that the criteria are used in a transparent and accountable process?

Answer: As committed during the July 2011 hearing, the EPA has already consulted with an independent panel of scientists on its third set of regulatory determinations (RD3). We specifically requested our expert reviewers to provide input on: the Agency's evaluation of the contaminants against the first and second Safe Drinking Water Act (SDWA) criteria [1) contaminant may have an adverse effect on the health of persons; 2) contaminant is known to occur or there is substantial likelihood that it will occur in public water systems with a frequency and at levels of public health concern]; whether the agency used the best available science to evaluate these criteria; and whether the process focuses on the contaminants of greatest potential public health risk.

Note that the third SDWA criterion (regulation of the contaminant presents a meaningful opportunity for health risk reduction for people served by public water systems) is subject to the sole judgment of the Administrator. The Administrator's decision of whether there is a meaningful opportunity to regulate a contaminant will take into consideration the input gathered from our expert panel and other relevant information.

We plan to publish the third round of regulatory determinations in the Federal Register and allow the public to comment on the process and factors used to identify and evaluate contaminants for this round of regulatory determinations. We will also make available the support document that provides more detail about the approach for RD3 and the input and advice provided by our expert reviewers.

Boxer 2. The Safe Drinking Water Act requires EPA to prioritize contaminants for regulation that present "the greatest public health concern," including for infants and children. The GAO found that the Office of Water "has not developed guidance for when and how to analyze the effects of drinking water contaminants on children ..." The GAO recommended that EPA create "approaches ... to evaluate [contaminants] health effects on sensitive subpopulations ... "

Will EPA develop such approaches and ensure their use in a transparent and accountable process?

Answer: The EPA agrees that it is important to evaluate health effects on sensitive populations such as infants and children, pregnant women, the elderly, and individuals with a history of serious illness. The EPA describes its consideration of the susceptibility of populations and life stages in the agency's health effects documents supporting each regulatory determination. To the extent that information is available in the literature defining the impacts on individuals with special susceptibilities, such as liver or kidney impairments, the information is provided to support the EPA's decision making. Immunocompetence is routinely considered in decisions concerning the occurrence of pathogenic organisms in source water and finished water. As described in the response to Boxer 1 above, the EPA is assuring a transparent and accountable consideration of effects to sensitive populations by requesting expert review and seeking public review and comment.

Boxer 3. Your testimony today states that EPA will consult with an independent panel of scientists when the Agency's is making certain determinations about whether a contaminant should be regulated under the Safe Drinking Water Act. I think that this is very welcome news.

Will EPA ensure that these scientific deliberations are transparent to the public?

Answer: Yes, when the EPA publishes its third round of regulatory determinations, we will publish the supporting documentation, which will include a summary of the input and advice provided by the expert panel of scientists.

Boxer 4. The GAO found that 9 of EPA's 20 determinations not to regulate contaminants involved data from monitoring activities that could not detect contaminants at levels that posed a health concern.

The GAO recommended that EPA develop a process to "reconsider whether to regulate a contaminant" that EPA has determined it will not regulate, and to "consider whether the agency needs to reevaluate any of its past determinations to not regulate [contaminants]."

Will EPA develop such a process, and is EPA considering whether to reevaluate any past decisions?

Answer: Yes, the EPA will reevaluate new health effects and occurrence information for contaminants that the EPA previously issued determinations not to regulate (i.e., a negative regulatory determination) in future Contaminant Candidate List (CCL) processes.

If new health or occurrence information becomes available on these contaminants, this information is considered to evaluate whether the contaminant should be listed on future CCLs and evaluated in future regulatory determinations processes.

Boxer 5. Deputy Administrator Perciasepe, the GAO found that in 2008, EPA's "Assistant Administrator [for Water] directed the staff to develop a determination to not regulate perchlorate and to support a specific exposure level as protective of all populations. This direction was outlined in an agreement between high-level officials at EPA and other federal

agencies ..." GAO also found that "key language" in the "notice appears to have been drafted by OMB rather than EPA." GAO has recommended that EPA include such agency comments in the public record in the future.

Will EPA agree to increase the level of public transparency in making these determinations?

Answer: The EPA will work to ensure the transparency, clarity and consistency of its regulatory determinations so the public can understand how the EPA came to its conclusions and so that the public can most effectively review and provide comment.

The EPA will identify for the public the substantive changes that occurred during the Office of Management and Budget (OMB) review and those changes made at the suggestion or recommendation of OMB. Specifically, the EPA will make available, in the public docket, documents that show the specific changes made to any regulatory determination notice (or other regulatory action) in the course of interagency review under Executive Orders 12866 and 13563 (either redline versions of the notice or before and after versions of the notice).

Boxer 6. In February 2011, Administrator Jackson reversed a Bush Administration decision not to regulate perchlorate with an announcement that EPA would move forward and develop a drinking water safeguard for this dangerous chemical. Perchlorate is a toxic contaminant that can harm children's development. I strongly support Administrator Jackson's decision.

Could you tell me if EPA is moving forward with the process of developing a drinking water standard for perchlorate? Also, can you assure me that the Agency will continue to use the best available science in crafting this standard?

Answer: The EPA is moving forward with the process to develop a perchlorate standard. The EPA is continuing to evaluate the science on perchlorate health effects and occurrence to develop a proposed drinking water standard for perchlorate. The EPA will also evaluate the feasibility and affordability of treatment technologies to remove perchlorate and will examine costs and benefits of potential standard levels. The EPA intends to publish the proposed regulation for public review and comment in 2013 and expects to promulgate a final regulation within 18 months of the proposal.

The EPA is committed to using the best available science and peer reviewed data in developing a drinking water standard for perchlorate. The agency will consult with our Science Advisory Board and with the National Drinking Water Advisory Council in developing the standard. In addition, the EPA will provide an opportunity for public comment on the proposed regulation, and will carefully evaluate and consider any new studies and data submitted by public commenters in developing a final regulation.

Boxer 7. In September 2010, EPA released a draft scientific assessment that found chromium-6 in drinking water is "likely to be carcinogenic to humans." In February 2011, EPA proposed to require monitoring for 30 contaminants, but did not include chromium-6 on that list. However, EPA asked utilities to voluntarily monitor for this heavy metal and has also asked for comment on whether monitoring should be mandatory.

Could you please tell me the status of EPA's consideration of whether to require monitoring for hexavalent chromium?

Answer: The EPA is in the process of developing the final Unregulated Contaminant Monitoring Regulation (UCMR 3) rule for the next cycle of unregulated contaminant monitoring and anticipates publication soon.

The EPA is giving serious consideration to the public comments we received on the question of including chromium 6 in the UCMR 3 rule. The EPA is also carefully considering what contaminant it would drop to stay within the 30 contaminant limit set by Congress if it does decide to require monitoring for hexavalent chromium. We will announce our decision when we publish the final rule.

Boxer 8. In September 2010, EPA released a draft scientific assessment that found the chromium-6 in drinking water is "likely to be carcinogenic to humans." The Agency has said it expects to finish this assessment in 2011.

Could you please tell me when in 2011 EPA will complete this assessment?

Answer: The EPA received the Final External Peer Review Report on the draft assessment and it was posted on the Integrated Risk Information System (IRIS) website (www.epa.gov/iris) in July 2011. The agency is currently considering the recommendations of the peer review panel, along with the comments we have received from the public, in determining the next steps for this assessment. A revised timeline for completion of the assessment will be available on IRISTrack (http://cfpub.epa.gov/ncea/iristrac/index.cfmin) in the near future.

Senator Whitehouse

Whitehouse 1. EPA's website links to a 2004 report entitled "PPCPs in the Environment: Future Research - Beginning with the End Always in Mind" (http://www.epa.gov/ppcp/pdf/daughton-needs.pdf). The report identifies the following "Needs and Gaps" in research about and policy responses to Pharmaceuticals and Personal Care Products (PPCPs):

- resource coordination;
- the sources and origins of PPCPs;
- detection of PPCPs in drinking water;
- methods for standardizing the chemical monitoring of water; and
- communication of risk and public outreach.

What advances have been made with regard to pharmaceuticals in drinking water in the areas listed in this report in the 7 years since it was published?

Answer: The EPA has been actively involved in efforts to identify compounds that are found in drinking water and surface water and determine appropriate responses to these issues. The agency continues to evaluate the potential risks to both human health and aquatic life that may be posed by these contaminants in water and is working with the relevant federal agencies.

The most comprehensive assessment of pharmaceuticals in drinking water occurred during the third Contaminant Candidate List (CCL3) evaluation process in 2008 (draft) and 2009 (final). In the CCL3 process, the EPA evaluated the potential adverse health effects of pharmaceuticals and their occurrence in public drinking water systems to determine if pharmaceuticals should be added to the list.

Since the publication of the draft CCL3 in 2008, several publications focused on the occurrence of pharmaceuticals in ambient water and drinking water were released. The EPA conducted additional data collection efforts to comprehensively review recent published information from the U.S. Geological Survey (USGS) and other sources to identify the best available occurrence information for pharmaceuticals. The EPA identified new occurrence data for 81 contaminants from 22 sources. The Agency also conducted additional literature reviews to identify the best available health effects information and identified data from sources including: the EPA Office of Pesticide Programs; the Food and Drug Administration (FDA) Center for Drug Evaluation and Research; the FDA Center for Veterinary Medicine; the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives; and the European Medicines Agency.

The EPA used the new data in the same process that was described in the draft CCL3 Federal Register notice to further evaluate pharmaceutical contaminants. Based upon this reevaluation with new data, the EPA concluded that one antibiotic (erythromycin) and nine hormones (17 alpha-estradiol, 17 beta-estradiol, equilenin, equilin, estriol, estrone, ethinyl estradiol, mestranol, and norethindrone), should be included on the CCL3 because these contaminants are known or substantially likely to occur in public water systems and may be appropriate for regulation.

In February, 2011, the EPA proposed the third Unregulated Contaminant Monitoring Regulation (UCMR 3). The EPA proposed to require monitoring for 30 contaminants (including 7

hormones) using an analytical method developed by the EPA. The EPA expects to promulgate the UCMR 3 in 2012 and begin data collection in 2013. The EPA uses data collected through UCMR to evaluate the frequency and level of contaminant occurrence, to support the determination of whether to regulate a contaminant, and to inform future contaminant candidate lists. In addition, the EPA is collaborating with the USGS to monitor finished drinking water and source water at more than 20 drinking water treatment plants across the nation to evaluate both the occurrence of contaminants of emerging concern in water (including pharmaceuticals) as well as the effectiveness of treatment on reducing levels. Results of this study are scheduled to be released in 2013.

Whitehouse 2. How does EPA plan to improve coordination internally with the many offices and laws (Resource Conservation and Recovery Act, Superfund, Clean Water Act, Safe Drinking Water Act) that regulate pharmaceuticals? How does the agency plan to improve coordination with other agencies such as the FDA?

Answer: The EPA continues to coordinate its efforts related to pharmaceuticals internally and externally. Within the EPA, we form agency-wide workgroups designed to allow all the EPA offices and regions to participate. These workgroups serve as the agency's internal means for coordination of the many offices and laws, including the Resource Conservation and Recovery Act, Superfund, the Clean Water Act, and the Safe Drinking Water Act, that may relate to the regulation of pharmaceuticals.

The agency also has ongoing collaborative efforts with the U.S. Geological Survey, the Department of Agriculture, the Office of National Drug Control Policy, and the Food and Drug Administration. In addition, the EPA has committed to establishing an interagency workgroup or other formal mechanism, as recommended by the Government Accountability Office (GAO) in its September 2011 report entitled "Action Needed to Sustain Agencies' Collaboration on Pharmaceuticals in Drinking Water," to collaborate and coordinate research on pharmaceuticals and, as appropriate, other contaminants in drinking water that present the greatest public health concern. To establish this mechanism, the EPA will build upon existing interagency collaborations with relevant federal partners and take steps identified by the GAO to: 1) define roles and responsibilities of existing and other relevant federal partners; 2) identify experts and other resources that each agency can bring to bear on the issue; and 3) develop a process for monitoring, evaluating, and reporting to the public the results of the collaborative research efforts. The EPA initiated this effort in late 2011.

Whitehouse 3. Is EPA planning to assess the risks to human health associated with long-term exposure to low concentrations of pharmaceuticals and the possible combined effects of pharmaceuticals, particularly on the general population and/or sensitive subpopulations?

Answer: The EPA assesses risks to the general population and/or sensitive subpopulations based on available occurrence and health effects data. As data becomes available for pharmaceuticals, the EPA plans to identify which, if any, of these contaminants require regulation or further research. The agency faces challenges in obtaining sufficient occurrence and health effects data on pharmaceuticals in drinking water to support these analyses and decisions. However, as mentioned in response to Whitehouse 1 above, the agency has proposed to collect occurrence information on seven hormones under the UCMR 3, and the agency is

working with the Food and Drug Administration and the U.S. Geological Survey to obtain this data.

Whitehouse 4. What preventative measures is EPA implementing or planning to implement, to address pharmaceuticals in drinking water - such as rational drug use and education of prescribers and/or the public to reduce disposal and discharges to the environment?

Answer: The EPA developed a consumer information sheet about how to properly dispose of household medications and has given step by step demonstrations (upon request) at local events. The information sheet was designed to educate the public as well as prescribers on ways to reduce the introduction of pharmaceuticals into the environment by following steps to properly dispose of unwanted or unused pharmaceuticals. The information sheet is available online at: http://water.epa.gov/scitech/swguidance/ppcp/upload/ppcpflyer.pdf.

However, disposal of household pharmaceuticals is a complex issue which requires collaboratio among federal agencies such as the Drug Enforcement Administration (DEA). The EPA is working with the DEA to inform the public about the environmental benefits of reducing discharges of household pharmaceuticals to the environment.

The DEA, in conjunction with state and local law enforcement agencies, conducts National Prescription Drug Take-Back Days. Nearly 4,000 state and local law enforcement agencies participated in these events, collecting more than 498 tons of pills. In October 2010, the President signed the Safe and Secure Drug Disposal Act, amending the Controlled Substances Act to allow the DEA to develop a process for people to safely dispose of their prescription drugs. While that process is under development, DEA continues to conduct Take-Back Days. The next Take-Back Day is scheduled on April 28, 2012. The EPA continues to work with the DEA to advertise these events. See http://www.deadiversion.usdoj.gov/drug_disposal/takeback/ for more information.

Whitehouse 5. Is EPA planning to undertake or fund any type of research to help waste water treatment facilities determine the practicalities of filtering out pharmaceuticals?

Answer: In 2010, the EPA released the results of an extensive literature review of published studies of the effectiveness of various treatment technologies for contaminants of emerging concern (CECs), including pharmaceuticals. The EPA also released a report that discusses some of the results of the literature search, including removals of specific CECs across common wastewater treatment technologies. The report discusses 16 of the more than 200 CECs present in the database, and the average percent removals achieved by full scale treatment systems that employ six of the more than 20 reported treatment technologies. Wastewater treatment plant operators, designers, and others may find this information useful in understanding ways to remove CECs from wastewater. The extent of contaminant removal varies greatly with the specific CEC and type of water treatment.

Whitehouse 6. Is EPA planning to or currently engaged in any monitoring of surface water, groundwater or wastewater effluent to assess possible occurrence levels and exposure to

particularly concerning pharmaceuticals, including estrogen compounds and cyotoxic chemotherapy drugs such as Mustargen?

Answer: The agency is sampling surface water at 154 sites across the nation for a suite of 54 pharmaceuticals, including estrogenic compounds, as part of the National Rivers and Streams Assessment survey. Data for this survey is scheduled to be released during spring 2012. Currently, the EPA is not sampling for cytotoxic chemotherapy drugs such as mustargen.

Senator Inhofe

Inhofe 1. The GAO report "Safe Drinking Water Act: EPA Should Improve Implementation of Requirements on Whether to Regulate Additional Contaminants" was made public at the Hearing. EPA agreed with two of the recommendations GAO made, yet at the hearing you appeared to agree with the majority of the report rather than just two recommendations.

Please describe how you will or will not be addressing each of the GAO's recommendations outlined in the report.

Answer: Please see the enclosed letter from the EPA Chief Financial Officer Barbara Bennett to Congressman Issa dated December 15, 2011, describing how the EPA is addressing each of the Government Accountability Office's (GAO) recommendations.

Inhofe 2. The National Academy of Sciences and the Agency for Toxic Substances and Disease Registry have concluded that the group of individuals most susceptible to harm from perchlorate exposure is fetuses of iodine-deficient pregnant women. In 2005, EPA accepted these conclusions. Does EPA continue to concur with those conclusions? If not, what is EPA's new position and what scientific support exists for EPA's new position?

Answer: The EPA continues to concur with the National Academy of Sciences' statements on page 27 of the 2005 report. "The thyroid hormones are critical determinants of growth and development in fetuses, infants, and young children. Thus, fetuses and preterm newborns constitute the most sensitive populations although infants and developing children are also considered sensitive populations. People who have compromised thyroid function resulting from conditions that reduce thyroid hormone production and people who are iodide-deficient also constitute potentially sensitive populations." Therefore, the EPA believes that fetuses, preterm newborns, infants, developing children, people with compromised thyroid functions and people with iodide deficiencies are all sensitive populations.

Inhofe 3. EPA has historically used a default body weight of 70 kg body weight and water intake of two liters per day to translate a reference dose to a drinking water concentration. These default values are already biased conservatively (low weighted adult in the numerator / large drinking volume in the denominator) and the RfD already has safety factors embedded into its derivation. Does EPA intend to change the use of these default values for all chemicals it evaluates for MCL/MCLGs? Why? How will EPA ensure any changes in default values are adequately vetted with the scientific community and transparently explained to the public?

Answer: The EPA has traditionally used a body weight estimate of 70 kg and daily consumption estimate of 2 liters per day to derive Maximum Contaminant Level Goals (MCLGs) for noncarcinogenic contaminants. These values have corresponded to the mean body weight for the general population and the upper (90th percentile) consumption rate estimates. However when there are "subpopulations that are identified as likely to be at greater risk of adverse health effects due to exposure to contaminants in drinking water than the general population," section

1412(b)(3)(C)(i)(V) of the Safe Drinking Water Act requires that the EPA analyze the effects of contaminant on these groups. The EPA will utilize the best available, peer reviewed science and data collected in accordance with accepted methods when evaluating the effects of contaminants on subpopulations likely to be at greater risk than the general population. Because infants' and children's body weight and drinking water consumption differ substantially from the general population, the EPA will utilize the best available, peer reviewed exposure data and analyses specific to these subpopulations since these groups have been identified as being likely to be at greater risk of adverse health effects. EPA also continues to explore the use of peer reviewed physiologically based pharmacokinetic (PBPK) modeling to assess exposure in sensitive subpopulations. The EPA will assure transparency of these analyses by publishing them for public review and comment with the proposed MCLG and drinking water rule.

Inhofe 4. The National Academy of Sciences has stated that inhibition of iodide uptake is the only effect of perchlorate exposure that had been consistently documented in humans from environmental levels of perchlorate exposure. ATSDR has stated that this effect has never been observed in humans. Can you point to any peer reviewed study in which inhibition of iodide uptake has been observed in human subjects from exposure to perchlorate in the environment?

Answer: The National Academy of Sciences (NAS) identified a mode of action model or pathway that leads from perchlorate exposure to inhibition of iodide uptake in the thyroid which in turn leads to changes in thyroid hormones and thyroid stimulating hormone (TSH) levels. The NAS stated "that the most reasonable pathway of events after sustained changes in thyroid hormone and TSH secretion would be thyroid hypertrophy or hyperplasia, possibly followed by hypothyroidism in people unable to compensate with an increase in thyroid iodide uptake. At that point, the pathway would diverge to two potential outcomes—metabolic sequelae (such as decreased metabolic rate and slowing of the function of many organ systems) at any age and abnormal growth and development of fetuses and children." The NAS clarified that "inhibition of thyroid iodide uptake is the only effect that has been consistently documented in humans exposed to perchlorate." Since the publication of the NAS report in 2005, a significant body of literature has been published that examines the association between perchlorate exposure and changes in thyroid hormones and TSH levels.

Studies demonstrating an association between perchlorate exposure in the environment and thyroid hormone or TSH perturbations include:

- Blount et al., 2006. Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. Environ. Health Perspect. 114:1865-1871;
- Steinmaus et al., 2010. Perchlorate in drinking water during pregnancy and neonatal thyroid hormone levels in California. J. Occup. Environ. Med. 52:1217-1524; and
- Cao et al., 2010. Goitrogenic anions, Thyroid-stimulating hormone, and thyroid hormone in infants. Environ. Health Perspect. 118:1332–1337.

Inhofe 5. EPA's Office of Inspector General has stated that "the nature of the public health issue [inhibition of iodide uptake] not only meets the requirements to be addressed by a cumulative risk assessment approach, but ... requires the use of a cumulative risk assessment approach to

accurately characterize the nature of the problem and to identify an effective and cost-efficient solution to the problem."

Will EPA conduct a cumulative risk assessment on perchlorate and the other chemicals that have the same mechanism of action?

Answer: Currently the EPA has no plans to conduct a cumulative risk assessment for perchlorate and other chemicals that may have the same mechanism of action. As indicated by the Inspector General's report on perchlorate, there are significant gaps in the research and data that need to be filled in order to inform a cumulative risk assessment. However, the EPA will qualitatively consider the Inspector General's conclusions and recommendations as it develops the proposed perchlorate standard.

The EPA is required to review and revise, as appropriate, its drinking water standards at least every six years. Any revision must maintain or improve public health protection. When there are sufficient scientific data to assess the cumulative risks of perchlorate and other contaminants, the EPA will review this information to evaluate whether any revisions of the National Primary Drinking Water Regulations are appropriate.

Inhofe 6. EPA's Office of Inspector General has concluded that of the three common goitrogens (thiocyanate, nitrate and perchlorate), perchlorate accounts for less that 1 percent of iodide uptake inhibition, while the other two chemicals account for more than 99 percent of iodide uptake inhibition. What is the meaningful opportunity for public health risk reduction through the regulation of perchlorate, given that more than 99% of the effect is related to other chemicals?

Answer: The EPA does not believe there are sufficient scientific data currently available to assess and characterize the combined and relative risk of these contaminants. The estimation technique the Office of the Inspector General used to estimate the amount of iodide uptake inhibition that would occur from perchlorate relative to thiocyanate, nitrate, and other substances is highly uncertain. The EPA concluded that perchlorate meets the SDWA's three criteria for regulating a contaminant:

- the potential adverse effects of the contaminant on the health of humans;
- the frequency and level of contaminant occurrence in public drinking water systems; and
- whether regulation of the contaminant presents a meaningful opportunity for reducing public health risks.

The science that led to this decision has been peer reviewed by independent scientists and public health experts, including the National Academy of Sciences. However, as noted above, the EPA will qualitatively consider the Inspector General's conclusions and recommendations as it develops the proposed perchlorate standard.

As described in the response to Inhofe 5 above, the EPA is required to review and revise, as appropriate, its drinking water standards at least every six years. Any revision must maintain or improve public health protection. When there are sufficient scientific data to assess the

cumulative risks of perchlorate and other contaminants, the EPA will review this information to evaluate whether any revisions of the National Primary Drinking Water Regulations are appropriate.

Inhofe 7(a). Is more recent water quality data available for perchlorate occurrence than the 2001-2005 data that EPA stated it has been using? Please provide any new data.

Answer: The EPA is continuing to collect and evaluate other drinking water occurrence data for perchlorate in addition to the data collected under the Unregulated Contaminant Monitoring Rule (UCMR) from 2001 to 2005 (see http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/data.cfm#ucmr2005). These additional data sources include:

- The California Department of Public Health (see http://www.cdph.ca.gov/certlic/drinkingwater/Pages/Perchlorate.aspx;
- The Massachusetts Department of Environmental Protection (see http://www.mass.gov/dep/cleanup/sites/percsour.pdf);
- The New Jersey Drinking Water Quality Institute (see http://www.state.nj.us/dep/watersupply/perchlorate_mcl_10_7_05.pdf; and
- The American Water Works Association (see http://www.awwa.org/files/Advocacy/PerchlorateOccurrenceReportFinalb02092005.pdf).

Inhofe 7(b). Additionally, in 2001-2005 the Colorado River contained up to about 10 ppb of perchlorate. According to a 2008 report by the Nevada Division of Environmental Protection, perchlorate levels in the Colorado River have decreased to less than 2 ppb. How is EPA taking this into account?

Answer: The EPA is evaluating the Nevada Division of Environmental Protection's perchlorate monitoring in the Lower Colorado River. We believe this information and other ambient water monitoring data can be informative with regard to the trends of perchlorate contamination in some source waters. However, we believe that samples collected from treated or finished water are more representative of the level of perchlorate in tap water because many systems combine water from multiple sources before treating and distributing that water to consumers. The EPA will utilize the best available, peer reviewed science and data collected in accordance with accepted methods when developing estimates of the number of systems that have perchlorate contamination and the populations exposed at various health reference levels.

Inhofe 8. The National Academy of Sciences, the Agency for Toxic Substances and Disease Registry, and EPA's Office of Inspector General have all concluded that a drinking water equivalent level of 24.5 ppb would be conservative and health protective. What peer-reviewed studies does EPA have that purport to demonstrate that this level is not sufficiently protective of human health?

Please provide a list of all peer-reviewed studies that EPA has that purport to demonstrate a causal link between exposure to perchlorate at drinking water levels of 24.5 ppb and below and adverse health effects.

Answer: In their 2005 Report, the National Academy of Sciences (NAS) concluded "that an RfD [Reference Dose] of 0.0007 mg/kg per day should protect the health of even the most sensitive populations." The NAS report does not convert the perchlorate RfD into a drinking water concentration. There are several factors that must be considered in identifying a health protective drinking water level based on the RfD. These include exposure through other sources (e.g., food) and selection of appropriate parameters to convert a dose per unit of body weight (the RfD) into a drinking water concentration. The NAS did not address these issues.

The EPA has not determined the level of perchlorate in drinking water at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety. In determining such a level, referred to in the Safe Drinking Water Act (SDWA) as a maximum contaminant level goal (MCLG), the agency will consider a variety of studies and factors. It is important to note that under SDWA, the agency must consider both effects to the general population and effects to sensitive subpopulations in identifying the health protective level. Once the MCLG is identified, the EPA must identify an enforceable regulatory standard, referred to as the maximum contaminant level (MCL).

Under SDWA, there are several steps for determining the MCL. First, EPA identifies a candidate MCL that is as close as "feasible" to the MCLG. The EPA evaluates feasibility by examining the available technologies that have been evaluated in the field. The EPA takes cost into consideration as part of the feasibility determination. The EPA also conducts a health risk reduction cost analysis which includes an evaluation of costs and benefits (both quantified and unquantified).

The EPA then makes a determination as to whether or not the benefits of the MCL justify or do not justify the costs based on this analysis. If EPA finds that benefits at the feasible level do not justify costs, EPA may set a less stringent MCL that maximizes health benefits at a cost that is justified. The EPA will lay out the full rationale for its proposed MCLG and MCL when it publishes the proposed rulemaking for public comment. It will also identify and include in the rule making docket all studies and research relied on for those determinations.

Inhofe 9. How does EPA determine conflicts of interest when putting together peer review panels for IRIS Risk assessments?

Answer: All draft human health assessments developed under the Integrated Risk Information System (IRIS) Program are subjected to rigorous, independent external peer review, most frequently by panels of scientific experts assembled by an external peer review service provider via a contractual agreement with the EPA. Panels assembled by the peer review service provider follow the procedures described on the IRIS website (http://www.epa.gov/iris/pdfs/Policy_IRIS_Peer_Reviews.pdf). Procedures include requiring panel members to submit periodic notifications certifying that no conflicts or potential conflicts of interest exist while reviews are ongoing.

Sometimes a panel of experts is convened by either the EPA's Science Advisory Board (SAB) or the National Research Council (NRC). The established process for independent peer review by

both the SAB and NRC and their respective procedures for determining conflicts of interest and any appearance of bias or lack of impartiality, are available on the SAB's website (http://yosemite.epa.gov/sab/sabproduct.nsf/WebSABSO/NominationExperts?OpenDocument) and on the National Academies' website (http://www.nationalacademies.org/coi/index.html).

Inhofe 10. Please provide the peer review comments in their entirety from the peer review panel for the Draft IRIS Risk Assessment for hexavalent chromium submitted to EPA.

Answer: The peer review report for the draft Integrated Risk Information System (IRIS) health assessment for hexavalent chromium is available on the EPA's website at: http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=221433

Inhofe 11. In a recent House Committee on Science, Space, and Technology hearing, there was discussion of EPA's use of overly conservative toxicity values along with overly conservative exposure scenarios. Does EPA feel these conservative assumptions result in characterizations of risk that accurately allows decision makers to address real world situations?

Answer: The EPA develops toxicity values and exposure scenarios specifically so that decision makers can address real world situations. They are not intended to be overly conservative; rather, they are based on the best available information. Where reliable information on specific toxicity and exposure parameters is not available, the EPA makes its best informed estimates of values or ranges for these parameters.

Sometimes, the choice of parameters in the absence of adequate data involves the use of default assumptions. The use of default assumptions was discussed and endorsed by the National Academy of Sciences (NAS) in "Science and Judgment in Risk Assessment" (1994), and the assumptions that the EPA makes follow the principles discussed by the NAS. Default assumptions do sometimes entail a degree of public health conservatism. The EPA is transparent about its default assumptions with decision makers and the public. The EPA believes that good risk management decisions should be based on a risk characterization which "integrates information from … the risk assessment and synthesizes an overall conclusion about risk that is complete, informative, and useful for decision makers." http://www.epa.gov/spc/pdfs/rchandbk.pdf). Further, the EPA subjects all of its risk assessments to external peer review and specifically asks peer reviewers to comment on the appropriateness of the major assumptions. This provides a check to ensure that decision makers have the best scientific information available.

Inhofe 12. Does EPA feel it is appropriate to set, based on application of EPA exposure assumptions, exposure levels below background concentration levels of ambient air, public drinking water sources, soil, or sediment? If so, how does EPA recommend State and local governments meet these levels?

Answer: The EPA recognizes that contaminants which may cause adverse health effects even at background levels present special regulatory challenges. There is no one size fits all answer to these situations. As EPA develops standards and regulations, it evaluates each contaminant

carefully, based on statutory requirements to promulgate regulatory requirements that best meet the goals of the authorizing statute to protect public health and the environment.

Inhofe 13. In May EPA announced reforms to the IRIS process as well as subsequent plans for further improvement as a direct response to the National Academy of Sciences Review of the Agency's Draft IRIS Assessment of Formaldehyde.

Does EPA believe it is addressing all of the NAS recommendations? Does EPA intend to document/demonstrate what specific activities have been put into place in order to implement the recommendations?

Answer: The EPA agrees with and is implementing all of the recommendations of the National Research Council (NRC), offered in their review of EPA's draft assessment of formaldehyde, that are related to the development of Integrated Risk Information System (IRIS) assessments. The EPA is addressing the NRC recommendations in a manner that is consistent with the NRC's "Roadmap for Revision" in Chapter 7 of the formaldehyde review report. Specifically, the NRC stated that "the committee recognizes that the changes suggested would involve a multiyear process and extensive effort by the staff of the National Center for Environmental Assessment and input and review by the EPA Science Advisory Board and others."

The IRIS Progress Report (http://www.epa.gov/iris/pdfs/irisprogressreport2011.pdf) outlines the EPA's plan to implement the recommendations and will be followed up with updates that document specific activities as they occur.

Most recently, the EPA requested public nominations of scientific experts for appointment to the EPA's Science Advisory Board Chemical Assessment Advisory Committee. The EPA will request advice from the committee as the agency implements the NRC recommendations.

Senator BOXER. I want to thank you very much, and say I think your response to this report is refreshing, that you are looking at it and embrace the notion of transparency, which is critical, science is critical.

In September 2010, EPA released a draft scientific assessment that found that chromium–6 in drinking water is "likely to be a carcinogenic to humans." The agency said it expects to finish this assessment in 2011. Can you give us the date when you will complete this assessment?

Mr. PERCIASEPE. That assessment we did last year was submitted for peer review. We just received a response from the peer reviewers and we are reviewing that. I would still think, depending on what depth we may need to go into, and I have to hedge the exact date here a little bit, based on the fact that we have not yet reviewed all the advice we got from the peer review. I think we are still on track to finish before the end of the year.

Senator BOXER. Good. I am going to assume that unless I hear otherwise. We have had our problems with chromium–6. I am really glad that you moved forward on it.

In February 2011, Administrator Jackson reversed a Bush administration decision not to regulate perchlorate with an announcement that EPA would move forward to develop a drinking water standard. I ask unanimous consent to place in the record comments from California urging you on in that regard.

[The referenced information follows.]

American Water Works
Association

The Authoritative Resource on Safe Water SM

July 26, 2011

The Honorable Barbara Boxer, Chair
The Honorable James M. Inhofe, Ranking Member
Senate Committee on Environment and Public Works
Washington, DC 20515

Dear Senators,

The American Water Works Association (AWWA) appreciates the committee's holding the hearing record open for two weeks following the July 12 hearing titled, "Oversight Hearing on the Environmental Protection Agency's Implementation of the Safe Drinking Water Act's Unregulated Drinking Water Contaminants Program." We request that this letter be made part of the hearing record.

The American Water Works Association (AWWA) is an international, nonprofit, scientific and educational society dedicated to the improvement of drinking water quality and supply. Founded in 1881, the Association is the largest organization of water supply professionals in the world. Our 55,000-plus members represent the full spectrum of the drinking water community: treatment plant operators and managers, environmental advocates, engineers, scientists, academicians, and others who hold a genuine interest in water supply and public health. Our membership includes more than 4,000 water systems that supply roughly 80 percent of the nation's drinking water. AWWA and its member utilities are dedicated to safe water.

With respect to the July 12 hearing, we would like to make the following key points:

1. **We believe the SDWA legislation of 1996 to be fundamentally sound.**

AWWA believes that Congress created a sound, science-based methodology for determining what new substances to regulate in the 1996 Amendments to the Safe Drinking Water Act (SDWA). In February 2011, we testified before this committee:

> "The SDWA was amended in 1996 to provide a scientifically sound and transparent method for selecting the appropriate substances for regulation and for selecting the appropriate maximum contaminant level for contaminants. We should allow the best available science, not the political process, to be the ultimate driver in regulatory decisions."

2. **With respect to the Unregulated Drinking Water Contaminants program provisions within the 1996 SDWA, there are success stories with respect to prior regulatory determinations about certain compounds.**

In many cases, substances of potential public health concern simply do not occur in drinking water sources and therefore, should be not be regulated. The following five substances in the

second regulatory determination in 2008 were found to have no occurrence in 3,479 systems across the U.S. under the first Unregulated Contaminant Monitoring Rule (UCMR1):

- 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene;
- 1,3-dichloropropene;
- 2,6-dinitrotoluene;
- *s*-ethyl-dipropylthiocarbamate; and
- Terbacil.

One substance (2,4-dinitrotoluene) had a single detection. Regulating these non-occurring substances would have expended scarce resources for EPA and water utilities without providing any health benefits whatsoever. Deciding to not regulate these six substances in the second regulatory determination was appropriate, and shows that the CCL process is working.

Additionally, EPA appropriately determined that regulating some other substances simply did not make sense as regulation would not provide a meaningful opportunity for health risk reduction. For example, the relative amount of sodium in drinking water is miniscule compared to food and sodium regulation in drinking water would not make a meaningful contribution to protecting public health. The same can be said for numerous other substances.

3. While the Safe Drinking Water Act is fundamentally sound, we agree there is a need to improve upon its implementation with respect to its Unregulated Drinking Water Contaminant provisions.

We acknowledge that the U.S. Environmental Protection Agency faces challenges in determining what substances to regulate in drinking water, particularly given the lack of health effects and occurrence data for many of the substances under consideration. But some of the challenges stem not so much from a lack of data as from problems with implementation. The GAO report "Safe Drinking Water Act: Improvements in Implementation Are Needed to Better Assure the Public of Safe Drinking Water" points to some systemic problems in the agency's implementation with which we largely concur.

First, we agree with the GAO that EPA needs to systematically implement the SDWA to better address substances that present the greatest potential for threats to public health. The agency and the public would benefit from criteria and processes for prioritizing such substances in its Candidate Contaminant List (CCL) and regulatory determination processes.

Second, as the comments above indicate, we concur with the GAO that greater transparency is needed in the regulatory determination process.

Third, concerning the lack of data for potential substances of concern in drinking water, AWWA has for years urged EPA to set a research agenda more closely aligned with and designed to support its regulatory agenda. Congress has a responsibility here as well, not only in oversight, but in ensuring that the agency has the resources needed to utilize or develop occurrence and health effects research.

4. **We remind Congress to consider any improvements in the Unregulated Drinking Water Program in the context of a comprehensive approach to protecting public health.**

While focusing on identifying and elaborating new standards for substances of emerging concern from the unregulated contaminants program, we remind Congress that treating water to the highest standards at a water treatment facility does not guarantee public health protection. It is vital that utilities also be able to convey drinking water to customers through a properly constructed and operated water infrastructure system. We appreciate that this committee has recognized the importance of federal support for investment in water infrastructure, including the State Revolving Loan Fund program. Another important issue is capacity development, as too many water providers struggle to meet increasingly complex regulatory requirements without adequate financial, technical, and managerial capacity.

Given all the above, we believe it is imperative that any improvements considered in the Unregulated Drinking Water program ensure that public resources be committed to those elements of the water supply system which have the most meaningful effect upon public health. There is a significant relationship between the regulatory agenda, infrastructure, and management capacity in that every dollar spent on treating for substances that don't represent a genuine public health threat is a dollar that could instead be used to rehabilitate or replace aging infrastructure and to build managerial capacity.

Once again, the Association appreciates the opportunity to comment on these critical matters and offers to work with the Committee further in advancing the treatment and provision of safe drinking water to the American public.

Sincerely,

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Senator BOXER. Perchlorate is a toxic contaminant that can harm children's development. I strongly support Administrator Jackson's decision. Could you tell me if EPA is moving forward with the process of developing a drinking water standard for perchlorate and can you assure me the agency will continue to use the best available science in crafting this standard?

Mr. PERCIASEPE. Yes, I can assure you on both counts, Madam Chairman. We are moving ahead on the process that is initiated by making the regulatory determination. And of course, we are going to be looking at all the best available science, as we had it, to make the right determination and as we will continue to improve the science that is available as we go through the regulatory process.

Senator BOXER. OK. Let me just say, given, I think, the criticisms that have come forward from the GAO, and the reality that so little has been done, the fact that you are moving on two contaminants out of so many, at least it is a start. So I am going to be watching very carefully to make sure that we don't get caught up in politics or anything else. This is about safety for our people. And we need to know. So I am going to be following those very, very closely.

Deputy Administrator, the GAO found that "EPA does not have criteria for identifying contaminants of greatest public health concern." They recommended that EPA develop "criteria to identify contaminants that pose the greatest health risks." Will EPA develop such criteria, in order to determine which drinking water contaminants should be regulated, and ensure that the criteria are used in a transparent and accountable process?

Mr. PERCIASEPE. What I mentioned in my opening statement is that EPA, it is sort of, things are going on in parallel here. In addition to the review that GAO has done, which has provided us meaningful input and good recommendations on transparency, we have also been in the process of trying to make those improvements at the same time. It is sort of, if you play ice hockey, it is sort of like changing the line on the fly, as they call it.

Senator BOXER. Oh, wait a minute, that is my husband's domain. He loves ice hockey.

Mr. PERCIASEPE. So you are familiar with that concept.

Senator BOXER. I can barely follow the puck, but I think it is exciting. But could you say what you said again, so I can ask him when I get home? Say it again, I am serious.

Mr. PERCIASEPE. Changing the line on the fly.

Senator BOXER. Changing the line on the fly. Thank you. I will check it out with him when I get home.

[Laughter.]

Mr. PERCIASEPE. I apologize for bringing a sports analogy in here.

But the point being that while GAO is looking at what we have done in the past, we are preparing the next round of the candidate contaminant list. And in doing that process, we have tremendously expanded this number of sources of science and data that we have looked at. And as I mentioned, we had almost 7,500 different contaminants we look at. I am doing this, because it is sort of like the top of a funnel. And as you go down the funnel to what you might recommend for a regulatory determination at the bottom, we are

building a very specific process that starts with the 7,000, looks at what we know about the severity of their health impacts, impacts on children or sensitive populations, occurrence information.

And we are moving down that list. We have publicly produced a document. We have had public hearings on it, about how we went from the 7,500 down to a smaller number. And now what we are focusing on 116 of them, each step of the way we have laid out a deliberate process.

Senator BOXER. OK, just to cut through, and I am going to give myself one more minute and give it to everybody else, the answer is yes?

Mr. PERCIASEPE. Yes.

Senator BOXER. The EPA is going to develop these criteria?

Mr. PERCIASEPE. Yes.

Senator BOXER. And you are going to make it public, and it will be transparent.

Mr. PERCIASEPE. And we are building it off of this.

Senator BOXER. OK. So my last question, and then I have a bunch I am going to ask you for in writing that are very important to me, the GAO found that 17 contaminants that have been on all three of EPA's lists of priority drinking water contaminants since 1998, and here we are, a long way from that, including RDX, or royal demolition explosive, which EPA has classified as a possible human carcinogen. The GAO raised concern with the potential lack of information that EPA has on some of these contaminants. We are talking about now a narrow list of 17 that have appeared on all these lists since 1998.

Will you agree to have EPA brief my staff on the status of the agency's information collection analysis activities for each of those 17 contaminants? And also at that time describe when EPA expects to consider these contaminants for a regulatory determination?

Mr. PERCIASEPE. Yes, of course. And I could add, I know that we are short on time, but a number of, a significant number of those are now on the final list that we are looking at in the current process. And a number of the other ones have been put on the unregulated contaminant monitoring list that David brought up in his testimony that we have expanded that list and will now get more data on them. So we are moving on pretty much all of those 17.

Senator BOXER. Thank you, and I will get you all these other questions I have, and turn to Senator Inhofe for 6 minutes.

Senator INHOFE. Thank you, Madam Chairman. And I would hope in the future, Mr. Administrator, if you are going to use a sports analogy, for my benefit, use either football or baseball, so we would understand.

[Laughter.]

Senator BOXER. Or soccer is good.

Mr. PERCIASEPE. Well, we have a spiritual soccer event earlier in the week.

Senator INHOFE. It could have been that the Chairman asked this question during her first minute, because I was distracted during that time. But I would like to know if there is anything further you could say on how the EPA is going to address the concerns that were raised by the NAS formaldehyde report, specifically the systemic concerns raised by the IRIS risk assessment process.

Mr. PERCIASEPE. In 2009, Administrator Jackson set in motion a number of improvements to the IRIS program to begin with. As you pointed out, maybe 10 years, where things had been allowed to perhaps lag for a while. So one of the things we did is improve some of the elements of the process. We have increased resources for it. And today our Assistant Administrator for the Office of Research and Development, Paul Anastas, is going to be announcing a next round of improvements to the IRIS process. And many of those improvements are on that same continuum we have been on now for a couple of years, and I think are very responsive to the advice we have gotten and that we appreciate also from the National Academy of Sciences.

So I am optimistic, Senator, that we are moving on that proper continuum to make sure that very critical, and I think you said it as well as anybody could in your opening statement, how important that IRIS process is to our program.

Senator INHOFE. OK, you are on that. As I said also in my opening statement, and you have heard me talk about this before, the concern that I have from my background as mayor of a major city in terms of the unfunded mandates. And again, I know other States have problems, too. I think Oklahoma certainly has great problems in our small communities.

Now, it is my understanding that the EPA section 319 program does help States in their implementation. Is this correct, in some of these programs?

Mr. PERCIASEPE. I am not completely familiar with that question.

Senator INHOFE. All right. Well, it is the EPA section 319 program. It does provide funds for some States and local entities to comply with some of these mandates. Is that correct?

Mr. PERCIASEPE. I think that—I am not familiar with 319. I probably know all the programs, but I don't know them by their numbers.

Senator INHOFE. OK, well, I was given the information that some $27 million was transferred from that program to the program that is sent to China and other countries.

Mr. PERCIASEPE. I am in the wrong law. It is in the Clean Water Act. Section 319 is in the Clean Water Act. I am familiar with that. That allows non-point source programs and State efforts on non-point source programs that can be used to do both source water protection and perhaps even wellhead protection in the proper context.

Senator INHOFE. Well, I think it is significant to bring up this point. Because you will remember, although you weren't in your current position, when Republicans were the majority and I chaired this Committee, I was concerned that people didn't have access to where a lot of the money was going from the EPA, from the Environmental Protection Agency. And so we set up a data base. And this has been pretty successful. And just the other day, Chairman Upton, of a comparable committee over there in the House, was talking about the $718,000 that went to China's EPA to assist with control of air emissions. And it goes on to talk about some of the other countries.

I just found this to be kind of mind-boggling that would take place. Is that incorrect? Because I know this would be the Assist-

ant Administrator for International Affairs. But that person does answer to you.

Mr. PERCIASEPE. I will have to look into that, Senator. I don't have any direct information at this particular moment on financial——

Senator INHOFE. OK, rather than just take it for the record, if you don't mind calling me back to see if I'm wrong in this. Because if I'm wrong and if Congressman Upton is wrong, we are doing a disservice by talking about this.

Mr. PERCIASEPE. I will make sure we get back to your office today.

Senator INHOFE. That is all I could ask for.

And I would say to you, Mr. Trimble, in your analysis, does EPA have all the statutory authority that they need to regulate contaminants of concern?

Mr. TRIMBLE. Sir, in our report and our recommendations, the deficiencies or the problems and the systemic problems we found were of a nature that we felt could be addressed strictly within the current authority. That is why our recommendations are to EPA, if we don't have a consideration for changing the statute, we believe that the EPA can take action quickly under existing authorities to define its policies and establish guidance, to interpret the statutes and implement them in such a way that it would be clear, consistent and provide a better product at the end of the day.

Senator INHOFE. All right. Thank you very much.

Thank you, Madam Chairman.

Senator BOXER. Thank you very much.

Senator Lautenberg.

Senator LAUTENBERG. Thanks, Madam Chairman.

Mr. Perciasepe, the New Jersey State legislature recently passed a ban on hydraulic fracking in my home State. But Pennsylvania and New York are moving ahead with fracking activities that could affect New Jersey's water supply.

Now, there is currently a loophole we are all familiar with in Federal law that exempts fracking operations from much of the Safe Drinking Water Act requirements. How does this loophole affect EPA's ability to prevent fracking contamination in one State from affecting the health of people in another, not unlike the winds that blow across our State from the west?

Mr. PERCIASEPE. Let me just say that on the movement of water on the surface of the, when the water, when the fracking fluids are used or whether there is produced water out of a well, when the water comes to the surface, those are under the jurisdiction, if they are discharged to waters of the United States, those are under the jurisdiction of the Clean Water Act. And there are requirements for how it would be pre-treated, or there would be State requirements on how it is pre-treated if it goes into a sewage treatment plant, or if it is reinjected into the ground, that would be covered by the underground injection and control program.

So on the surface, I think, Senator, the Clean Water Act would apply. The exemption is the regulation of the injection of the fracking fluids into the ground to conduct that operation. Those are not regulated, or that action is not regulated under the under-

ground injection and control program, which is in the Safe Drinking Water Act.

Senator LAUTENBERG. Well, a few months ago, I asked you about the air quality impacts of natural gas drilling. And in your testimony you said that natural gas drilling would lead to "an increase in emissions of volatile organic compounds and nitrogen oxide." But there was no offer of any concrete steps that EPA is taking to reduce these emissions.

Is EPA taking any action now to address emissions from natural gas drilling?

Mr. PERCIASEPE. I will mention two briefly, Senator. First, just in the last several weeks, EPA, the U.S. Department of Agriculture and the Department of Interior signed an MOU on how we would conduct air quality analyses on leasing programs, either in the national forests or on the Bureau of Land Management's land, which again, mostly is going to be out west. But we have now a multiagency agreement on how we will analyze the air impacts and what requirements might be placed on the leases that are put forward.

We just recently conducted that analysis on a lease that the Department of Interior, through the Bureau of Land Management, made in the Grand National Buttes in Utah. And those requirements, and the company there was Anadarko, those requirements are very progressive and forward-leaning. And the company is moving forward in that.

Senator LAUTENBERG. When will we get something specific there to move on this? The studies are being done.

Mr. PERCIASEPE. Right. So those requirements would be placed on those leases, and they would have to meet those standards. The second thing, though, that I want to mention, is EPA is also working on what under the Clean Air Act is called new source performance standards. So as a new well is produced or developed, it would be required to reduce the amount of volatile organic compounds that would be emitted while the well development is being conducted.

So those proposals will be out later this year. And they will be in many ways similar to what we are requiring companies to do when they lease on Federal land.

Senator LAUTENBERG. The concerns are real. But the pace seems fairly slow in doing something about it.

Mr. PERCIASEPE. I appreciate that very much.

Senator LAUTENBERG. In addition to individual pollutants in drinking water, people are often exposed to multiple contaminants. The interaction with each other can cause some health effects. Mr. Trimble, how might EPA consider the health effects of the combination of chemicals when they set drinking water contaminant limits?

Mr. TRIMBLE. I think that is an important issue. I think it goes to the heart of our recommendations, which is that the EPA needs to have guidance and policy and criteria to explain how such issues are considered in their decisionmaking. For example, when they make a decision, at the end of the day they need to explain was it considered, how was it considered. If they have a policy and it is an exception to that policy, they need to transparently and clear-

ly explain all the factors that led them to the outcome that they arrived at.

So I think we don't, we are not a science agency, so we are not making recommendations on how they should or shouldn't do these things. But from our standpoint, you need to establish the road map for the staff to follow in getting there. And then that road map not only guides your operations throughout your execution of this program, but at the end of the day, when you explain your decisions, it allows you to clearly articulate the basis for your decisions and credibly defend them.

Senator LAUTENBERG. Chairman, there are so many questions that arise from this review, so the record is, I understand, will be kept open.

Senator BOXER. Absolutely, the record is open. And Senator Inhofe wanted to make a request.

Senator INHOFE. Yes, I want to make a unanimous consent request, I didn't know that hydraulic fracturing was going to be coming up. I would like to make an observation that the first hydraulic frack job was done 60 years ago in my State of Oklahoma and there has never been a documented case of ground contamination. I have a statement from some, about 15 other States, all the other States where this is a concern. I would like to have this put into the record, that states the same thing about their States as I stated about Oklahoma.

Senator BOXER. Absolutely. That will be put in the record.

[The referenced information follows.]

STATEMENTS ON HYDRAULIC FRACTURING SUBMITTED BY STATE REGULATORS

ALABAMA: Nick Tew, Ph.D., P.G. Alabama State Geologist & Oil and Gas Supervisor President, Association of American State Geologists
"There have been no documented cases of drinking water contamination that have resulted from hydraulic fracturing operations to stimulate oil and gas wells in the State of Alabama."

ALASKA: Cathy Foerster Commissioner Alaska Oil and Gas Conservation Commission
"There have been no verified cases of harm to ground water in the State of Alaska as a result of hydraulic fracturing."

COLORADO: David Neslin Director Colorado Oil and Gas Conservation Commission
"To the knowledge of the Colorado Oil and Gas Conservation Commission staff, there has been no verified instance of harm to groundwater caused by hydraulic fracturing in Colorado."

INDIANA: Herschel McDivitt Director Indiana Department of Natural Resources
"There have been no instances where the Division of Oil and Gas has verified that harm to groundwater has ever been found to be the result of hydraulic fracturing in Indiana."

KENTUCKY: Kim Collings, EEC Director - Kentucky Division of Oil and Gas
"In Kentucky, there have been alleged contaminations from citizen complaints but nothing that can be substantiated, in every case the well had surface casing cemented to surface and production casing cemented."

LOUISIANA:
James Welsh Commissioner of Conservation Louisiana Department of Natural Resources
"The Louisiana Office of Conservation is unaware of any instance of harm to groundwater in the State of Louisiana caused by the practice of hydraulic fracturing."

MICHIGAN: Harold Fitch Director, Office of Geological Survey (OGS) Department of Environmental Quality
"There is no indication that hydraulic fracturing has ever caused damage to ground water or other resources in Michigan. In fact, the OGS has never received a complaint or allegation that hydraulic fracturing has impacted groundwater in any way."

OKLAHOMA:
Lori Wrotenbery Director, Oil and Gas Conservation Division Oklahoma Corporation Commission
"You asked whether there has been a verified instance of harm to groundwater in our state from the practice of hydraulic fracturing. The answer is no."

TENNESSEE: Paul Schmierbach Manager Tennessee Department of Environmental Conservation
"We have had no reports of well damage due to fracking."

TEXAS: Victor G. Carrillo Chairman Railroad Commission of Texas
"The practice of reservoir stimulation by hydraulic fracturing has been used safely in Texas for over six decades in tens of thousands of wells across the state."

SOUTH DAKOTA: Fred Steece Oil and Gas Supervisor Department of Environment and Natural Resource
"In the 41 years that I have supervised oil and gas exploration, production and development in South Dakota, no documented case of water well or aquifer damage by the fracking of oil or gas wells, has been brought to my attention. Nor am I aware of any such cases before my time."

WYOMING: Rick Marvel Engineering Manager Wyoming Oil and Gas Conservation Commission & Tom Doll Oil and Gas Commission Supervisor Wyoming Oil and Gas Conservation Commission
"There have been no documented cases of groundwater contamination from fracture stimulations in Wyoming."

PENNSYLVANIA: John Hanger Department of Environmental Protection Secretary "It's our experience in Pennsylvania that we have not had one case in which the fluids used to break off the gas from 5,000 to 8,000 feet underground have returned to contaminate ground water."

NEW YORK: Taury Smith, State Geologist "Those are exaggerated problems (of contamination); each incident wasn't the result of hydro-fracking. There were incidents of groundwater contamination near frack sites, but they were unrelated."

Senator BOXER. And now we turn to Senator Boozman.

Senator BOOZMAN. Thank you, Madam Chair.

I think one of the things that is so important as we deal with the agencies and things, especially in dealing with issues that really do affect our health, economic matters in the sense that, trying to conform to some of the things that we come up with literally will cost billions of dollars in some instances, that you have to have confidence in the system. Mr. Trimble, you talked about the agency performing tests where they were doing testing and maybe, I will put it in my terms and you can correct me, maybe they were testing for one part and then the test itself was only good to two parts. Sometimes only good up to 2,200 parts, and then making decisions based on that.

Can you comment further on that?

Mr. TRIMBLE. Sure. In the decisions that they made in, I believe it was 2003, 2008, to not regulate about 20 contaminants, I believe in 9 of those the testing data that the program had developed was not at a level sufficient to detect the contaminant at the point of concern. So exactly to your point, it wasn't robust enough to test it at the level of concern.

That raises lots of issues. But what we talk about in the report, when we reviewed the determination in getting to the issue of the credibility, is that the impact of that limitation is either completely not addressed in the documentation explaining the decision, the determination decision, or it is buried. There could be other reasons why, maybe it is best available, maybe you have other data. But the issue and the basis for reaching a decision regarding sort of a big piece of this as well. So you have the problem with the testing program, but then you also have just the lack of explanation of, well, OK, you had this problem, how did you get to this point.

Senator BOOZMAN. You also have the problem, too, in the sense that you can have a false sense of security, can't you, in the sense that a test was supposedly performed and everything was OK?

Mr. TRIMBLE. Absolutely. In four of the decisions, they did not acknowledge that there was this limitation or the impact of that limitation on their conclusion that there was no health issue.

Senator BOOZMAN. So is that currently going on? Has that gone on recently?

Mr. TRIMBLE. Our review looked at the 20 decisions since the 1996 amendments. And in nine of them, we identified this issue. So that, I believe the last group we looked at was 2008.

Senator BOOZMAN. Can you comment on that? Is that currently still a problem, or in the recent years been a problem?

Mr. PERCIASEPE. We feel, and I mentioned in my opening comments that we have modified our approach to looking at the unregulated contaminant monitoring that we are allowed to do. We are making sure that the testing that is done there, and these are the suite of contaminants that we are concerned about but that are not currently regulated, and we are trying to determine if their occurrence, as David just mentioned, is prevalent enough that it would be of concern. But you have to measure for the right level. And we are confident that we have modified our processes to be able to do that.

Senator BOOZMAN. Mr. Trimble, you talked about the three criteria for acting and went on to say that some of the people in the EPA, when interviewed, said there are no bright lines, that we will know it when we see it. Was that from scientists, or were these political people? How does anybody that is familiar with the scientific process, how do you deal with statements like that?

Mr. TRIMBLE. I would have to check who it was. But I think it was more the regulatory office. And the issue of the discussion was not seeing the need or not recognizing the need for guidance or a road map in terms of how you interpret this. It was sort of a we know it when we see it kind of decision. Again, the problem as you have pointed out is the credibility that leads to, where you can have, you may see it, I may see it, somebody else may see something different, you can have changes over time, changes between people. And that is the issue I was trying to get at.

Senator BOOZMAN. Yes, sir?

Mr. PERCIASEPE. And I also want to point out that we are equally concerned with this and are happy to get this advice. I think how to solve this problem is probably open to a little bit of interpretation. GAO has laid out, I think, a thoughtful way to do it. We are in the process of trying to do it differently this time with developing our current list of contaminants that we are concerned about. We have gone through a very deliberate process that dissects those three large categories that are in the Act, like is there an adverse health effect. Well, we are looking at potency, we are looking at sensitive populations, and we are screening down to that. We also have to look at the occurrence data. We are looking at both regional as well as national data.

The last step, and as I testified, the last step in the judgment of the Administrator is there a meaningful opportunity for a health risk reduction, well, we are proposing now, and what I have asked the water program to look into is the staff working on those first two, developing a report and then having that report subject to a peer review which asks these questions. And then the Administrator would have in front of her both the staff report on the adverse health effects and the occurrence or substantial occurrence data across the Country or regionally, and that judgment would be able to be also enhanced, by that peer review of that work that was done.

So in that final step which is in the judgment of the Administrator, they would have both of those pieces of information together. And I feel that if we get that peer review to ask some of these questions, because there are so many different kinds of contaminants, that they will be able to see if the staff properly did the integration. However, we are learning from doing it differently this time, and we definitely want to have the criteria, as the Chairwoman mentioned, be clearly laid out.

Senator BOOZMAN. Thank you.

Mr. TRIMBLE. I would just note, we have not obviously reviewed the latest thinking and proposals. I am encouraged by the direction. I note a couple of things. One, the triaging of the contaminants of concern into the candidate list down to now the current list has about 116 contaminants. That is, EPA has indicated that is a prioritization, and it is. It is a first step.

But 116 is not enough of a prioritization to guide your direction from there. In 2009, the Science Advisory Board also had a report encouraging EPA to prioritize.

Senator BOOZMAN. Thank you, Madam Chair.

Senator BOXER. Thank you very much.

Senator Cardin?

Senator CARDIN. Thank you, Madam Chair.

Mr. Trimble, first, let me thank you for this work. I think it is extremely important. Mr. Perciasepe, I appreciate the attitude of EPA to make sure we get this right.

It is very concerning when we know that we have had lists now of unregulated contaminants. And there has been inconsistency in the methods used to determine whether there should be regulation. Our bottom line is to protect the public health of the people of this Nation. They rely upon us to determine whether water is safe to drink. And we have a responsibility to make sure that in fact is being done.

What was most disconcerting about the GAO report, you showed the listing of new contaminants for regulation, lacked a procedural safeguard, that there were different variations of assessment based on the chemical involved. So Mr. Perciasepe, let me just ask you first, to what extent do you think the variation of the assessment procedures from one chemical to another affects the reliability of the EPA determinations?

Mr. PERCIASEPE. Well, Senator, there may be radionucleides, there could be viruses, there could be bacteria or other protozoans as well as chemicals. So all of them have different ways you have to analyze them. I think that there is a commonality of approach, though, when you start with a large list and you start looking at which one of those things on that large list has the most potential health effects, whether it be sensitive sub-populations or whether it be to the general population. And you keep narrowing that list down. David used triage, I would call it some deliberate screening.

And I agree that you get that list down to 116, that is not enough. Because this is really the precursor to deciding what your priorities are to actually do a regulation on. So this is not itself a regulation, this is just deciding what to regulate.

And I think it is important that we do the best job we can, publicly and with criteria and with disclosure on how we got that, including peer review. And then from that list, the Administrator has to decide. And we think the list will be smaller than 116.

In this instance, we think it will be down to 30 or maybe less. And that is what we will ask the outside reviewers to say, do we have that list right, and did we ask these right questions. And then that is what would be in front of the Administrator, to make that determination.

Senator CARDIN. Can we learn something from perchlorate, in that it was included in the first uncontaminated chemical monitoring rule, so at least many years ago, there was at least some concern about it? Perchlorate is a toxic component of rocket fuel that may disrupt the production of thyroid hormones which are particularly sensitive for fetuses in young children. I know that the Administration is moving forward on perchlorate. Can you at least give us the status of where we are on that, and whether there is

a lesson to be learned as to why we did not investigate this issue earlier?

Mr. PERCIASEPE. Well, obviously it went through the process that I just described as it was designed in many respects in the detail that GAO looked at. But since that time, we have re-looked at the science there. We looked at not just modeling of these physiological effects, but also the actual exposures to children and pregnant women. That forms some of the key basis for the Administrator making that determination that it is required to go through the regulatory process.

So we are starting that regulatory process now, once that determination is made.

Senator CARDIN. What is the timeframe?

Mr. PERCIASEPE. I have to say, with the proposal and then the final, it is probably going to be at least 2 years.

Senator CARDIN. And one of the questions I would raise, first of all, I think that is too long. But I understand the procedures you have to go through. You used the funnel example as to how many contaminants are listed, and you need to make a priority judgment as to which are the greatest at risk and what can be regulated effectively for public health.

Perchlorate to me was too high up in that funnel, it should have been at a lower part of that funnel at an earlier stage, which I think is what the GAO report really, this is an example of the inadequate testing that was done earlier dealing with perchlorate. So I hope we have learned that lesson to get it down to the level of regulation earlier. And I would hope that you would work with us to streamline the regulatory process, because if the risk factors are to a vulnerable group, this requires us to act in a more expedited way.

Mr. PERCIASEPE. I agree, and I think, I mean, I have worked with some members here today on that Safe Drinking Water Amendment in 1996, when I was in the government back in the 1990's. We deliberately wrote into the law that the Administrator had to make a decision. They couldn't just keep analyzing, there had to be a decision made. I think that what has happened is that the transparency of that decision and the criteria used to get to that point, and the peer review of the staff and science that was being used in that final step by the Administrator to decide yea or nay on a regulatory determination, it is that point that got, I think, bent out of shape in the last time the determination was made. And I believe that having a peer review at that particular moment will, you never solve every problem, but it will, I think provide more transparency to why and if the Administrator is making decisions based on the most current science.

Senator CARDIN. Thank you very much.

Senator BOXER. Thank you, Senator.

Senator Udall, welcome.

Senator UDALL. Thank you, Senator Boxer, and thank you for holding this important hearing.

First, on behalf of the residents of northern New Mexico, I would like to say thank you to the EPA, represented here by Deputy Administrator Bob Perciasepe. The EPA provided really important emergency air monitoring resources, including their ASPECT plane

to monitor air quality during the largest forest fire in New Mexico's history, which threatened Los Alamos National Laboratory and many other areas in New Mexico. Those air monitoring results, the independence of them, the State looked at things, EPA looked and the lab had their numbers.

But these air monitoring results we have seen so far show no unusual pollution beyond what would be in a normal forest fire, and EPA's independent presence on the ground and in the air was of great value, I think, in providing the public with credible information. So I thank you for that.

On the topic of today's hearing, several witnesses, including the GAO, observed that the EPA has not fully taken into account the impact of unregulated drinking water contaminants on children's health, I know Senator Cardin mentioned this earlier, as required by the Safe Water Drinking Act and the agency's policy on children's health. And perchlorate, in particular, is one that concerns me in the decisionmaking on perchlorate. Perchlorate is an unregulated contaminant found in many drinking water systems in the west, which is home to many of our Nation's major military facilities. New Mexico's communities are proud to host our Nation's armed forces, but all communities, I think, deserve clean drinking water.

Just as with forest fires, we are counting on EPA to provide accurate, independent scientific information and take the appropriate action.

So let me first start with Mr. Trimble on this issue of children's health and the overview of the work you did. Could you please describe the safe Drinking Water Act and the EPA policy requirements on considering children's health impacts in drinking water standards, and then two, describe your report's findings on how EPA met these requirements, and three, describe how recommendations you made relate to those children's health requirements.

Mr. TRIMBLE. Sure. First, the requirement to prioritize contaminants of greatest concerns includes a provision that prioritization should include impacts on children and other sensitive sub-populations. So that is sort of square one, that is part of the prioritization. And again, it goes to the recommendation we have regarding how you establish that priority list.

In observing how that has been implemented with EPA and our work in doing those determinations has not integrated specific risk assessments for children as part of its determinations. In 2003, in the determinations they made to not regulate additional contaminants, it acknowledged that it did not have the methodology to address the impact on children, but did not then make any other explanation as to how they were going to figure out whether this was protective of children's health.

In 2008, they made additional determinations where they acknowledge that these contaminants had an effect on children's health, but they did not explain in their analysis how that potential risk was addressed in their analysis, and how their conclusion that these contaminants did not need regulation was protective of children's health.

In perchlorate, similar concerns were raised about children's health. In fact, that is one of the reasons why the decision was reconsidered, was concerns about uncertainty as well as the impact on children. What I would note in that regard is while the EPA, from my understanding, has decided to regulate perchlorate, it is not clear at this point whether the additional analysis it did in reconsidering its decision for perchlorate will be carried forward in future determinations. So in other words, are they going to integrate that analysis and the impact on children in future determinations. It is still a little unclear from what we have been given whether that would happen.

Senator UDALL. Thank you.

Mr. Perciasepe, could you please respond to what we have heard from the GAO on children's health and what will EPA do to implement GAO recommendations in this area?

Mr. PERCIASEPE. We feel that we are required by law under the Safe Drinking Water Act to consider the sensitive populations. I won't read it, I have it sitting here in front of me, but I can guarantee you that it is in the law and it is clearly laid out, and the GAO has pointed this out clearly, that we should be making a clear analysis. It depends on what the health effect of the substance is. I would have to say some may not have an impact on children in the same way.

But it doesn't negate the need to do it. So we would be agreeing with that assessment that we need to be doing that. That is going to be also part of our overall screening process of even when it gets down to being looked at as a hard contaminant. For instance, we have a contaminant on our current list, a subset of that 116 we mentioned earlier, for instance, strontium, which has a very important negative effect on bone development, particularly in children. So that is one that is on the list particularly because of children.

Senator UDALL. Thank you. Thank you, Madam Chair.

Senator BOXER. Thank you. And Senator Udall, thank you for all your work on children's health. This is so critical, because we have a responsibility to them.

I just want to thank the panel very, very much, GAO for your clarity. I think this report was very good. And I think it should be embraced by everyone, because what you are basically saying is transparency in these decisions, move quicker on these decisions, and get it done. And I applaud the EPA for your response, which is, we hear you. I just want you to know, we are watching on chromium–6 and perchlorate. I am looking forward to that briefing.

And by the way, anybody else who wants to hear the briefing on these 17 contaminants that have been looked at since 1998 and nothing has been done, we are going to have a briefing. We just might do it as an open briefing, but I will let you know about that.

Mr. PERCIASEPE. OK, we are ready.

Senator BOXER. Well, we appreciate it so much. We really do appreciate it. And even your sports analogies which drew our interest.

[Laughter.]

Senator BOXER. Thank you very much.

Mr. PERCIASEPE. I will remember to use football the next time.

Senator BOXER. Football, baseball, soccer, that is fine. It is good. Women's soccer in particular would be good.

[Laughter.]

Senator BOXER. OK, panel two. Dr. Lynn Goldman; A.W. "Butch" Araiza; Dr. Joseph Cotruvo; Dr. Steven Patierno; and Dr. Jeffrey Griffiths. And each of you will have an opening of 5 minutes. And at that point, I will ask you to put the remainder of your statement in the record. So if you could summarize.

We are going to start with Lynn Goldman and we are going to move down the table, if that is OK. So Dr. Lynn Goldman, welcome, American Public Heath Association, Dean of the School of Public Health and Health Services, and Professor of Environmental and Occupational Health, George Washington University. We are very delighted that you are here, thank you.

STATEMENT OF LYNN GOLDMAN, MD, MPH, AMERICAN PUBLIC Health ASSOCIATION, DEAN OF THE SCHOOL OF PUBLIC HEALTH AND HEALTH SERVICES AND PROFESSOR OF ENVIRONMENTAL AND OCCUPATIONAL Health AT THE GEORGE WASHINGTON UNIVERSITY

Dr. GOLDMAN. And thank you very much. It is my pleasure to testify about the Environmental Protection Agency's implementation of the Safe Drinking Water Act's unregulated drinking water contaminants program on behalf of the American Public Health Association.

I have been a member of the APHA for almost 20 years. I am pleased to represent them today. The APHA is dedicated to protecting all Americans and their communities from preventable, serious health threats and assuring community-based health promotion and disease prevention activities and preventive health services are universally accessible in the United States. APHA has long advocated for strong environmental health laws.

Safe drinking water is essential to public health, and our Nation's drinking water supply is one of the safest in the world. Along with the EPA, our Nation's State and local health departments also play a critical role to ensure the safety of our drinking water. Additionally, there are more than 170,000 public water systems providing water.

While most of our drinking water does not violate EPA standards for maximum contaminant levels, the APHA is concerned over a number of public health issues. First, we are concerned about the decline in Federal resources to State to improve drinking water infrastructure. In fact, the final 2011 continuing resolution, and I have heard possibly the 2012 budget as well, is cutting significant amounts of money, hundreds of millions of dollars, from the EPA's Safe Drinking Water State Revolving Fund.

Second, we are concerned about unregulated contaminants. Tens of thousands of chemicals are on the market, but the vast majority of these will never appear in finished drinking water. Those that are in drinking water should be regulated by EPA so the public can be assured that levels are safe.

A number of specific chemicals have been of concern to the APHA, including perchlorate, chromium–6, trichloroethylene and other volatile organic chemicals, and a number of pesticides and

disinfection products found in drinking water across the Nation. APHA expected that the 1996 Safe Drinking Water Act amendments would have increased the number of chemicals that were regulated by EPA. Unfortunately, EPA did not adopt new drinking water safeguards for chemicals after that law was passed. And a recent study by the Environmental Working Group reported that hundreds of unregulated contaminants are found in our drinking water systems.

Why do we need to have MCLs? Minus the establishment of MCLs and health-based MCL goals, how are we to know that the levels of chemicals in drinking water are safe? In response, several States have undertaken drinking water regulation of unregulated contaminants, for example, the State of California regulating perchlorate, chromium–6, TCE, several VOCs and pesticides. But only national standards can assure safe drinking water across our entire Country.

Third, we are concerned that EPA standards not only protect the average adult but also adequately protect infants and children, the frail elderly and those with weakened immune systems. As a pediatrician, I am particularly concerned about infants less than 6 months of age who receive drinking water in infant formula. These infants consume five times more drinking water per body weight than adults. They therefore have greater exposure to any substance in drinking water than adults would. And yet, we also know that at these life stages that they may be more sensitive, less able to metabolize, detoxify and excrete toxic substances, as well as more sensitive to developmental effects, effects on growth and development.

The 1996 Safe Drinking Water Act amendments directed the EPA to protect the general population, as well as those significant sub-populations. This authority needs to be exercised by the EPA in the regulation of unregulated contaminants.

In conclusion, APHA suggests a number of ways that EPA could strengthen the regulation of chemicals in drinking water. EPA does need to implement the 2009 National Academy of Sciences report, Science and Decisions Advancing Risk Assessment, especially the recommended changes in risk assessment design, dose response modeling, uncertainty analysis and analysis of cumulative risk. Cumulative risk is not only the risk of multiple chemicals that might act in the same way, but also the risks that people have in other exposures in daily life that create public health threats like nutrition, exposure to smoke, other carcinogens.

EPA needs to develop MCLs for unregulated toxic chemicals and pesticides that are present in drinking water so that the public can be confident of safety. And third, EPA needs to strengthen its efforts to protect vulnerable populations such as infants and children and pregnant women from risks of drinking water contaminants.

Thank you for the opportunity to testify this morning, and I am happy to answer any questions.

[The prepared statement of Dr. Goldman follows:]

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Protect, Prevent, Live Well

Testimony of Lynn Goldman, MD, MS, MPH
Representing the American Public Health Association
Oversight Hearing on the Environmental Protection Agency's Implementation of the Safe Drinking Water Act's Unregulated Drinking Water Contaminants Program
Senate Committee on Environment and Public Works
July 12, 2011

Chairman Boxer, Ranking member Inhofe and members of the Committee, it is my pleasure to testify about the Environmental Protection Agency's Implementation of the Safe Drinking Water Act's Unregulated Drinking Water Contaminants Program on behalf of the American Public Health Association.

My name is Lynn Goldman. I am Dean of the School of Public Health and Health Services at The George Washington University School of Public Health and Health Services. I am a pediatrician and an epidemiologist. I have done research on health impact of environmental contaminants and am a member of the Institute of Medicine. From 1993-98, I served as Assistant Administrator for the Office of Chemical Safety and Pollution Prevention at the US Environmental Protection Agency (EPA). Prior to joining the EPA I worked for eight years in public health with the California Department of Health Services. I also am a member of the Board of Trustees of the Environmental Defense Fund. I have been a member the American Public Health Association (APHA) for almost 20 years, and I am pleased to represent APHA at today's hearing regarding the public health implications of the Safe Drinking Water Act (SDWA). APHA is the nation's oldest and most diverse organization of public health

professionals in the world, dedicated to protecting all Americans and their communities from preventable, serious health threats and assuring community-based health promotion and disease prevention activities and preventive health services are universally accessible in the United States. APHA has long advocated for strong environmental health laws that adequately protect the health of the public from environmental hazards and we appreciate the opportunity to testify today on the public health implications of our nation's safe drinking water laws.

The SDWA, last amended in 1996, is the nation's primary law to protect the public's health from harmful contaminants in the nation's drinking water supply. While the EPA administers the law and sets standards, the states have been delegated primary authority for enforcing drinking water programs. Our nation's state and local health departments also play a critical role in working with state drinking water regulators to ensure the safety of our drinking water.

Safe drinking water is essential to public health. According to the EPA, there are currently more than 170,000 public water systems that provide water to most Americans, and our nation's drinking water supply is one of the safest in the world. Since its enactment, the SDWA has made significant improvements to the quality of the nation's public water supplies. An overwhelming majority of Americans receive their drinking water from sources that do not violate EPA's standards for maximum contaminant levels. At the same time, the public health community remains concerned over a number of issues that continue to provide risks to the public's health, including unregulated contaminants and decreasing federal resources to states to improve drinking water infrastructure. In fact, in the final 2011 continuing resolution cut more than $400 million (or 30 percent) from the FY 2010 level for EPA's Drinking Water State Revolving Fund.

In addition, APHA believes more must be done to protect vulnerable populations that are more susceptible to exposures, including infants/children, pregnant women, immune-suppressed individuals, and the elderly when setting drinking water standards.

There are weaknesses in federal statutes and regulations governing the safety of drinking water, and a number of EPA standards are being currently being reviewed and revised. In some instances, contaminants are not regulated, such as perchlorate and a number of pesticides. EPA standards may protect the average adult but may not adequately protect infants and children, the frail elderly, and those with weakened immune systems. Moreover, EPA standards are established to protect health while considering the water treatment costs and availability of clean-up technology.

Numbers of Contaminants Unregulated

At the time the SDWA was amended, APHA expected that this would result in an uptick in the numbers of chemicals regulated since SDWA gave EPA more flexibility to establish priorities for regulation of contaminants based on those that present the greatest public health concern, including for pregnant women, infants and children. Unfortunately, this expectation was not fulfilled, and my review of EPA's drinking water regulations at CFR 40 part 141 finds that EPA has not adopted any new drinking water safeguards for chemicals since enactment of the 1996 law – neither to assure the safety of unregulated contaminants nor to change regulations for previously-regulated chemical contaminates. Tens of thousands of chemicals are on the market but the vast majority of these will never appear in finished drinking water. Those that are in

drinking water should be regulated by the EPA so that the public can be assured that levels are safe. According to a December 2009 report issued by the Environmental Working Group, hundreds of additional unregulated contaminants have been found in our drinking water systems. Minus the establishment of clear Maximum Contaminant Levels (MCLs) and health-based MCL Goals, how are we to know that these chemicals in their water are "safe"? A number of specific chemicals have been of concern to the APHA and the public health community in states, including: perchlorate, chromium VI, trichloroethylene (TCE) and other VOCs, and a number of pesticides and disinfection products that are found in drinking water across the nation. Unfortunately, this situation has caused several states to undertake drinking water standard setting on their own, ahead of EPA action, most notably, the State of California, which has established standards for perchlorate, chromium VI, TCE and several other VOCs, and, a number of pesticides. While APHA believes that states are responsible for taking such steps to protect the public's health, we would prefer to see national standards that can effectively assure safe drinking water for all of the country.

SDWA and Children, Pregnant Women, and Other Vulnerable Populations

In 1995, APHA developed a policy that recommended that all environmental policy, legislation, and regulation protect children and we have long advocated that environmental health standards need to protect vulnerable populations. It is important to recognize that, as we state in that policy, "children are uniquely vulnerable to environmental exposures because they are in a dynamic state of growth, with many vital systems such as the nervous, immune, and respiratory systems not fully developed upon birth" and to understand "that children can have greater

exposures to environmental toxins". In the case of drinking water, children drink more water and consume more water indirectly through food, per body weight, than adults. According to EPA estimates, considering all drinking water intake, infants less than 6 months of age consuming drinking water in infant formula, per body weight consume five times more drinking water than adults consume in both drinking water and food. This means that they have greater exposure to any substance, microbial or chemical, that might be present in that drinking water. At various life stages – during pregnancy, *in utero*, in childhood, in old age, and during serious illnesses -- people may have different abilities to metabolize, detoxify, and excrete certain many toxic substances, thus rendering them more vulnerable to health effects. Moreover, rapid growth and development *in utero* and in early childhood puts children at particular risk for exposure to environmental toxins that may disrupt normal developmental processes, and result in permanent irreversible damage. Traditionally EPA's environmental health regulations have been based on data primarily from research on healthy adult humans or animals and do not take into consideration the unique exposure patterns and sensitivities of children, pregnant women, the elderly and those with chronic diseases.

In the 1996 SDWA amendments, Congress specifically directed the EPA to regulated unregulated contaminants that are at greater risk of adverse health effects due to exposures than the general population, including infants, children, pregnant women, the elderly, and individuals with a history of serious illness, or other subpopulations recognized to be of greater risk. Likewise, in establishing MCL goals, EPA was to protect the general population as well as these significant subpopulations. This authority has been exercised by EPA to craft stronger

regulations for microbial contaminants like *cryptosporidia* in drinking water, but not for addressing unregulated chemical contaminants.

Conclusions and Recommendations for Improvement

APHA suggests a number of ways that EPA could strengthen the regulation of chemicals in drinking water:

1. In all of its risk assessment efforts, EPA needs to implement the 2009 National Academy of Sciences report *Science and Decisions: Advancing Risk Assessment*. This report recommended changes in how EPA designs risk assessments, models dose response for hazardous substances, and considers uncertainty in risk assessment. In particular it concluded that EPA needs to address the issue of cumulative exposures. Exposures to drinking water contaminants occur within a context of multiple exposures to other contaminants in drinking water, foods, household environments, and air. They also occur within contexts such as significant subpopulations who have other health problems that may contribute such as poor nutrition and exposure to tobacco smoke. All of these issues need to be considered in an overhaul of EPA's risk assessment processes, including for drinking water.
2. EPA needs to more strongly weigh evidence that chemicals and pesticides are present in drinking water. Given that there are no mechanisms for systematic collection of information about levels of unregulated chemicals in drinking water, those toxic chemicals that are occurring frequently need MCLs so that they will be monitored and the public can be assured that levels are safe.

3. Each and every one of us was, at one time, a fetus or a child; have been or may become (if we are female) pregnant; has suffered from or may have a chronic illness; and (if we are lucky) may become elderly. These aren't "subpopulations"; they are life stages. EPA needs to strengthen its efforts to assure that vulnerable populations are protected as required by law.

Thank you for the opportunity to testify this morning and I am happy to answer any questions.

Goldman Follow Up Question Responses

Questions from Senator Barbara Boxer

1. Without specific guidance, the EPA will not be able to develop a science-based approach that can be applied consistently to ensure that children are protected from contaminants in drinking water considering multiple developmental stages.

   Yes, this is the type of action that APHA has in mind.

   Yes. The agency should take a proactive approach to identification of drinking water contaminants that are most likely to be hazardous to pregnant women and children, review those and, where appropriate, establish protective standards.

2. Yes, this effort is consistent. APHA is concerned that the Agency move forward quickly, especially in the case of chemicals with similar toxicity that are likely to co-occur (for example, pesticides in surface water supplies).

3. First, a six kg, six-month-old baby is about one-tenth the size of a 70 kg adult male. If the baby consumes five times more water per body weight than the adult, that means the infant is receiving a 50 times higher dose (per body weight) of any contaminant in the water.

   This has obvious practical importance, especially in the case of contaminants that are particularly toxic during development.

Questions from Senator James Inhofe

1. I have testified as an expert witness on two occasions. One I was a witness for a plaintiff and once I was a witness for the defendant. In the first instance, compensation was received by my university; in the second instance, I received some compensation.

2. I have never served on a peer review panel for any EPA risk assessments.

3. No, I do not. I believe that the drinking water infrastructure in the US in many areas is aging, and that over the years, the EPA and Congress will need to continue to focus attention on assuring that the American people have safe, plentiful, and affordable supplies of drinking water.

Senator BOXER. Thank you, Dr. Goldman. I think that cumulative risk issue is key. I thank you for bringing it up.

And it is my pleasure to introduce Mr. A.W. "Butch" Araiza, General Manager of the West Valley Water District in the Inland Empire, my State. It has been my pleasure to work with them. They face some tough issues and we look forward to hearing from him.

STATEMENT OF ANTHONY ARAIZA, GENERAL MANAGER, WEST VALLEY WATER DISTRICT

Mr. ARAIZA. Thank you, Chairman Boxer and Ranking Member Inhofe and members of the Committee. It is an honor to be asked to testify today about the West Valley Water District's success in removing perchlorate from groundwater.

I would first like to communicate my profound thanks to Senator Boxer. Over the years, you and your staff have been key in helping us secure Federal funding for our remediation efforts in helping encourage the Environmental Protection Agency and the Department of Defense to aid in treatment of contaminated water in our region. West Valley Water District's success is very much your success as well. Thank you.

By way of introduction, I have been employed by the District for the past 48 years. I worked my way up through the ranks to the position of General Manager nearly 16 years ago. In 2002, the District became the lead agency in Inland Empire Perchlorate Task Force, which is responsible for coordinating the investigation and response to the serious perchlorate contamination crisis in our area.

West Valley Water District was founded almost 60 years ago. Today, we serve almost 20,000 homes and businesses. Our staff and board of directors are dedicated to protecting, safeguarding and delivering clean water to our customers at the lowest reasonable price. The necessity to innovate was presented clearly by our need to remediate the effects of widespread perchlorate pollution in the inland empire. The source of that pollution is a 160-acre industrial site in Northern Rialto that used to be a U.S. Army munitions depot. The State of California has set a limit of perchlorate of 6 parts per billion.

But the plume migrating from the site has led to levels as high as 800 parts per billion. This has forced West Valley Water District and other water providers in the area to shut down or otherwise restrict the use of at least 22 groundwater production wells, representing more than half the region's water supply. Additional groundwater production wells may need to be shut down as pollution continues to migrate. We have no choice other than to remediate. Groundwater is the main source of drinking water in the region and alternative sources are not reliable. Estimated costs for cleaning up the whole problem would be in the hundreds of millions of dollars and could take up to 30 years.

Meanwhile, remediation poses a significant burden to the ratepayers in one of the most economically disadvantaged regions in the Nation. Facing this crisis and lacking the vast resources required to solve it, West Valley Water District has looked to innovative solutions. We were fortunate to find that the Department of Defense's Environmental Security Technology Certification Pro-

gram, ESTCP, and its extremely capable executive director, Dr. Jeffery Marqusee. Under the leadership of Dr. Marqusee, ESTCP is demonstrating the most innovative and cost effective technologies that address DOD's high priority environmental requirements. Our work with ESTCP is a win-win partnership, providing our customers clean water while collecting data that the ESTCP uses to advance cost-effective technology.

The District's perchlorate remediation efforts start in 2003, utilizing technology known as ion exchange. We are currently running five ion exchange systems, one of which is cutting edge and was made possible by the ESTCP. Ion exchange systems work by trapping perchlorate and resin, and our experience has proven them to be safe and reliable.

With the support of ESTCP and the State of California, we have recently broken ground on the first facility to use naturally occurring biological process to treat drinking water. Bioremediation allows natural process to break down perchlorate and other harmful chemicals in the water. Bioremediation has proven safe and effective, does not use harmful chemicals and requires less annual maintenance than other methods. Our project will provide clean tap water to our customers and opportunities for ESTCP to save the Department of Defense significant amounts of money at sites they may have liability for across the Country.

We are proud to provide a venue for demonstrating this pioneering green technology. It should not surprise you that West Valley Water District is not going to stop working toward the future, now that the bioremediation project is underway. We anticipate this project will become a component of a much larger undertaking to fully restore the region's groundwater supplies. The District is currently working with the United States Environmental Protection Agency, the State of California and parties responsible for the contamination to develop a plan for final phase of groundwater clean-up. This is a lot of work yet to be done, and we are looking for more innovative solutions, just as we have been for the past 60 years.

Thank you for this opportunity to testify to the Committee, and I would be happy to take any questions.

[The prepared statement of Mr. Araiza follows:]

Testimony of Anthony Araiza
General Manager
West Valley Water District

before the
US Senate Committee on Environment and Public Works

"Oversight Hearing on the Environmental Protection Agency's Implementation of the Safe Drinking Water Act's Unregulated Drinking Water Contaminants Program"

July 12, 2011

Chairman Boxer, Ranking Member Inhofe, and Members of this Committee, it is an honor to be asked to testify today about West Valley Water District's success in removing perchlorate from groundwater as part of our mission to deliver safe and affordable drinking water to residents of the Rialto-Colton area. I am pleased to be able to share our experience with the Committee because we believe we have been part of the solution.

Before I introduce myself or explain what we've been doing, I would first like to communicate my profound thanks to Senator Boxer. Over the years, you and your staff have been key in helping secure federal funding for our remediation efforts and helping encourage the Environmental Protection Agency and the Department of Defense to aid in the treatment of contaminated water in the region. West Valley Water District's success is very much your success as well. Thank you.

By way of introduction, I have been employed by the District for the past 48 years. I worked my way up through the ranks to the position of General Manager nearly 16 years ago. In 2002, I became the lead member of the Inland Empire Perchlorate Task Force, which is responsible for coordinating the investigation and response to the serious perchlorate water contamination crisis impacting approximately 22 drinking water wells and 500,000 people in the Inland Empire.

Background on WVWD

When the predecessor to West Valley Water District started almost 60 years ago, it served a very rural area and few water customers. Today we serve almost 20,000 homes and businesses, and our service area encompasses more than 29 square miles. Despite the many changes, our staff and Board of Directors have maintained a constant dedication to protect, safeguard, and deliver water to our customers at the lowest, reasonable price. We serve as fiduciaries, for the public trust. We do so not only by sticking to the tried-and-true, but also by looking for new innovations – for example, we recently re-vamped our web site, developed a Facebook page and a started a Twitter feed to help ensure the very best service to our customers.

Perchlorate

The necessity to innovate was presented clearly by our need to remediate the effects of widespread perchlorate pollution, which affects the groundwater aquifers on which the West

Valley Water District, the Cities of Colton and Rialto and the Fontana Water Company rely to provide drinking water to the citizens of the Inland Empire. The source of that pollution is a 160-acre industrial site in northern Rialto that used to be a U.S. Army munitions depot. The State of California has set a limit for perchlorate of six parts per billion, but the plume migrating from this site has led to levels as high as 800 parts per billion. Perchlorate contamination has forced the West Valley Water District and other water providers in the region to shut down or otherwise restrict the use of at least 22 groundwater production wells in the area, representing more than half of the region's water supply. Additional groundwater production wells may need to be shut down in the near future as the pollution continues to migrate.

We have no choice other than to remediate. Groundwater is the main source of drinking water in the region, and the basin's water levels are approaching an historic low. Alternative sources such as the State Water Project are not reliable. The estimated cost of cleaning up the whole problem could be in the hundreds of millions of dollars and take up to 30 years. The necessity for remediation in the region poses a significant burden for ratepayers in one of the most economically disadvantaged regions in the nation.

ESTCP

Facing this crisis, and lacking the vast resources required to solve it, West Valley Water District has looked for innovative solutions. We were extremely fortunate to find the Department of Defense's Environmental Security Technology Certification Program (ESTCP) and its extremely capable executive director, Dr. Jeffrey Marqusee. ESTCP is DoD's environmental technology demonstration and validation program, which promotes innovative technologies. Under the leadership of Dr. Marqusee, the ESTCP program has succeeded in achieving its goal "to identify and demonstrate the most promising innovative and cost-effective technologies and methods that address DoD's high-priority environmental requirements." We work with ESTCP's Environmental Restoration Program, which has been a win-win partnership. The District and our customers benefit through cost-effective clean water, and the data collected helps the ESTCP's goal of advancing science and technology to provide the Department of Defense (DoD) with effective management and restoration of its contaminated assets. Dr. Marqusee has been a problem solver who has helped significantly in moving forward with solutions.

Remediation

The first stage of the District's perchlorate remediation efforts have utilized a technology known as ion exchange. We are currently running 5 ion-exchange systems, one of which is a cutting-edge design that was made possible by the ESTCP. Ion exchange systems work by trapping perchlorate in resin, and our experience has proven them to be safe and reliable.

With encouragement and support from ESTCP and the State of California, we have entered a second stage of remediation by breaking ground on the first bio treatment facility to utilize a naturally occurring biological treatment process to treat contaminated drinking water supplies. Bioremediation allows natural processes to breakdown perchlorate and other harmful chemicals in the water. After this natural process occurs, the water is sent through a traditional water treatment process before it enters the drinking water system. Bioremediation is proven safe and

effective, does not use harmful chemicals, and requires less annual maintenance than other methods.

The system under construction will utilize a fluidized-bed reactor, and we hope to combine it with an additional treatment system which will feature a fixed-bed reactor. Because the two systems will treat water from the same underground source, side by side, our partnership with the ESTCP will provide a unique opportunity to test the effectiveness, sustainability, and costs of each system – a tremendous benefit that will help future clean-up efforts throughout the country. In other words, our project will provide opportunities for the Department of Defense to save significant amounts of money at sites they may have liability for across the country. We're proud to be a part of the solution and to provide a venue for demonstrating this pioneering "green" technology.

I would be remiss if I did not acknowledge that these projects would not have gotten off the ground with out considerable help from others in addition to Senator Boxer and Dr. Marqusee. Senator Feinstein and Congressman Baca have been tremendous allies. The State of California has provided $10 million in grants, and the State Water Resources Control Board has also contributed crucial resources.

Future

It should not surprise you that West Valley Water District is not going to stop working towards the future now that the fluidized bed reactor construction is underway. As I just mentioned, we anticipate that this project will become a component of a much larger undertaking to fully restore the region's groundwater supplies. The District is currently working with the United States Environmental Protection Agency, the State of California and parties responsible for the contamination to develop plans for the final phase of groundwater cleanup. There is a lot of work yet to be done, and we are looking for more innovative solutions – just as we have been doing for the last 60 years.

Summary

The staff and Directors of West Valley Water District are fiduciaries for the public trust, dedicated to serving our 20,000 households clean water through efficient service. We are focused on the future and we're looking for innovative solutions to the challenges that confront us. Although the perchlorate crisis has presented a significant challenge in our region, we are working tirelessly to be part of the solution, not only for our ratepayers, but also – thanks to the ESTCP – for everyone else who is or may be affected by perchlorate in the future. With the help of our elected representatives including Senator Boxer, we are providing new technologies that can remove perchlorate safely and cost efficiently. This is a win-win collaboration for West Valley Water District, our customers, the DoD, and all Americans who rely on the delivery of clean, safe and affordable drinking water.
Thank you.

West Valley Water District

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November 2, 2011

Senate Committee on Environment and Public Works
Attn: Jonathan Aronchick
410 Dirksen Senate Office Building
Washington, DC 20005

Dear Mr. Aronchick:

Enclosed please find my answers to the questions submitted by Senator Inhofe for the hearing record for the July 12, 2011, hearing entitled, "Oversight Hearing on the Environmental Protection Agency's Implementation of the Safe Drinking Water Act's Unregulated Drinking Water Contaminants Program." As requested, I have reproduced the questions with my responses.

I hope my answers are helpful to the Committee as it continues its work on this important topic. Please contact me if you have any further questions or need any additional information.

Sincerely,

WEST VALLEY WATER DISTRICT

Anthony "Butch" Araiza
General Manager

1. The Committee hears a lot from the smaller drinking water utilities around the country about issues complying with unfunded drinking water mandates such as new rules or additional testing. How does West Valley Water District respond to these mandates and challenges?

   West Valley Water District is accustomed to managing the difficulties of providing a clean, safe water supply while keeping costs reasonable for our ratepayers. Our organization believes that we should work to make certain our water is as clean as possible for our users because it is the right thing to do, not simply because we are required to meet state or federal standards. The State of California has set a limit for perchlorate of six parts per billion. We have been able to manage this challenge by working to implement cutting-edge water treatment technologies, like the bioremediation project I mentioned in my written and oral testimonies. Chairman Barbara Boxer, Senator Dianne Feinstein, and others have been successful in helping us develop innovative partnerships with federal and state agencies to test and help fund new technologies.

2. What are some of the challenges that utilities face in responding to media reports about unregulated contaminants?

   Media reports can easily lead to concerns about the safety and quality of water supplies. West Valley Water District believes that public outreach is vital to ensuring our users have accurate information about their water supply, and we have employed an aggressive outreach strategy. As mentioned in my written testimony, we maintain a very informative website, we are on Facebook, and we even have a Twitter feed. Through these and other more traditional mediums – informational mailings, public meeting, etc. – we work to educate our customers about information that can come through traditional media, the internet, and other sources. We strive to ensure our users know exactly what is in their water and the steps West Valley Water District is taking to protect the water supply.

3. Are you supportive of a drinking water regulatory process that relies on science to help guide decision making? Do you think the current SDWA provides a clear, transparent, science-driven process for making decisions regarding drinking water regulation?

   West Valley Water District supports a sound scientific process as it relates to the regulatory framework for groundwater supplies. Few federal statutes are perfect, and the Safe Drinking Water Act is not without room for improvement. As Ranking Minority Member Inhofe pointed out in his opening statement, the National Academy of Sciences has recently pointed out that the Environmental Protection Agency's (EPA) risk

assessment system is flawed. And, as Chairman Boxer pointed out in her statement, a recent Government Accountability Office report indicated that the EPA's process for developing standards for unregulated drinking water contaminants failed to use the best available science. West Valley Water District would be happy to participate in discussions with the committee if our experience could be helpful in finding ways to address those issues.

Senator BOXER. Thank you very much, and we welcome our next speaker, Dr. Joseph Cotruvo, it is Joseph Cotruvo and Associates, LLC, Water, Environmental and Public Health Institute. Welcome, sir.

STATEMENT OF JOSEPH A. COTRUVO, PH.D., PRESIDENT, JOSEPH COTRUVO AND ASSOCIATES

Mr. COTRUVO. Thank you, Chairman Boxer and Senator Inhofe. I appreciate the opportunity to come and talk to you today.

My name is Joseph Cotruvo. I have spent more than 35 years in public health matters. The doctorate is in physical organic chemistry. I was the first Director of EPA's Drinking Water Standards division back about 35 year ago when the law was passed, and spent a number of years there. In the course of that, I was involved in developing many of the existing regulations and in fact, many of the methodologies that are used for regulating.

The regulations are very comprehensive. We will talk about more of them later. Currently I work internationally on water quality and health. I do some non-U.S. Government assistance on regulatory activity and water quality. I serve on a number of advisory committees, including World Health Organization's guidelines for drinking water quality, which is actually what the rest of the world, outside the United States, uses for establishing its drinking water safety.

I am also a member of the D.C. Water and Sewer Authority board of directors here. I just want you to know, I am not representing any organization or institution. These are my personal thoughts, conclusions, and I am not receiving any compensation for this presentation.

On the topic of drinking water quality and safety, almost all community water systems in the United States produce safe drinking water, including Washington, DC, although some may not believe that. It is a daunting task, because we are dealing with 65,000 water supplies, 25 persons up to millions. And it is a very complex job that is a multi-faceted job. EPA has a role, States have a role, and of course, it is all underpinned by those guys that are out there every day, 24–7, producing that water at the tap.

Overall, I think EPA has done a generally good job of implementing the Safe Drinking Water Act. The record shows that reported waterborne infectious disease outbreaks have declined since the implementation of the law. And the portion of outbreaks due to distribution systems and infrastructure has increased. So overall, it has declined, but that part has gone up. That tells us very clearly that the problem is infrastructure, it means that water going in from the plant better than the water that it coming out at the tap.

So fixing that and maintaining is, I think, really the greatest public health challenge that we have to face here, and the greatest public health concern.

The current regulations are very comprehensive. They cover a number of substances, 81 MCLs, microbial contamination, which basically covers all microbial contaminants, radionucleides all are covered. There is corrosion control, there are some specific items also. But the numbers even belie the reality in that they actually

cover much, much more than just those numbers, because all those regulations, or many of them in fact have a cascade of other substances that are protected against by the regulation that is in place.

Regulations have to be protected. They are designed to be protective. They have certain safety factors built in, and that is what we expect. But it is of course important that they have to be realistic, and they have to be appropriate for the kind of risk involved, and the significance, and in fact, the strength of evidence that there is really a risk involved. That is why part of the task that EPA has is so difficult. Because with all of these new analytic methods, as you heard from the GAO, methods keep getting lower and lower. We used to talk about parts per million, now parts per billion, now parts per trillion, and next year it will be parts per quadrillion, probably.

So what all that means is we are finding more and more substances. It doesn't mean that there are more there than were there 5 years ago or 10 years ago or whatever. It is just we are finding them now. Invariably, when one is measuring something at levels that are that low, it is really hard to conceive that there is some significant risk from exposure at that minute level.

Just to put some things in perspective a little bit, I have a couple of items here mentioning reports on perchlorate and pharmaceuticals. I will just read you the report that came this year from the UNFAO, WHO, JECFA Committee: "The estimated dietary exposures of 0.7 micrograms per kilogram per day, highest, and 0.1 microgram per kilogram per day, mean, including both food and drinking water, are well below the provisional maximum tolerable daily intake. The committee considered that these estimated dietary exposures were not of health concern."

So there are differing points of view that one has to deal with in this business. And that makes all of our jobs and EPA's very difficult.

[The prepared statement of Mr. Cotruvo follows:]

Senate Committee on Environment and Public Works
Chair, Senator Barbara Boxer

Oversight Hearing on USEPA's Implementation of the Safe Drinking Water Act's Unregulated Contaminants Program

July 12, 2011

Joseph A. Cotruvo, Ph D
President: Joseph Cotruvo and Associates
Washington, DC

Good morning Senator Boxer and members of the committee.

My name is Joseph Cotruvo. I have spent more than 35 years engaged in public health and environmental matters. My doctorate is in Physical Organic Chemistry. I was the first Director of EPA's Office of Drinking Water Criteria and Standards Division after passage of the Safe Drinking Water Act, where I had a role in developing many of the existing drinking water regulations and regulatory methodologies, and later in the Risk Assessment Division in Pollution Prevention and Toxics. Those regulations included comprehensive microbial protection, trihalomethanes, radionuclides, Surface Water Filtration, corrosion control, Volatile Synthetic Organic chemicals (VOCs) and numerous other organic and inorganic chemicals. Currently, I work internationally in water quality, health and technology including helping to establish a drinking water regulatory program in Singapore, and on desalination and health related issues and epidemiology in the Middle East, and on development of small water treatment technologies for home and small systems applications, as well as on some basic toxicology studies. I serve on several independent advisory groups dealing with drinking water safety issues, including many years with the World Health Organization's Drinking Water Quality Committee, which last week issued the 4th edition of the Guidelines for Drinking Water Quality. These are the benchmarks that most of the world outside of the US try to apply to define drinking water safety. I am also a member alternate of the Washington DC Water and Sewer Authority.

I am not here representing any organization or institution; these are my personal thoughts and conclusions, and I am not receiving any compensation for this presentation.

- **Drinking Water Quality and Safety**

Almost all public community water systems in the United States provide safe drinking water --including Washington, DC-- contrary to what some believe, and that is no small accolade and accomplishment when dealing with the 60,000 entities providing drinking water, which range in population from as few as 25 persons to millions. The quality and safety of drinking water in the United States is very good and almost always more consistent and better than the limited number of nations that have substantial drinking water regulatory and enforcement programs in place. We always strive for improvement. US policies and processes for safe drinking water provision have clear leadership standing in the world and many if not most of the existing methodologies for assessing and regulating drinking water quality and safety were developed here first.

The EPA's and the states and water suppliers' implementation of the Safe Drinking Water Act of 1974 and the later amendments has significantly improved the safety of drinking water in the United States. It is indeed a joint effort that is implemented 24/7 by the often underappreciated professionals in the national drinking water delivery community that we all rely on to actually produce and deliver safe water. The comprehensive protection program includes regulation, advice, monitoring and system assessments and operations, supported by an enforcement program, and significant financial support from the federal government. The underpinning of it all is that there is that cadre of dedicated water professionals who manage and operate those water systems.

The record shows that reported waterborne infectious disease outbreaks have declined since implementation of the SDWA began, but the portion of outbreaks due to distribution system infrastructure deficiencies has been increasing. That is a clear indication that the water coming out at the tap isn't always as good as the treated water leaving the plant. It is a symptom of our ageing infrastructure. Fixing and maintaining infrastructure and reducing leaks and contamination in distribution is where our priorities now belong.

- **Current Regulations**

The 1986 amendments to the SDWA specified a long list of chemicals to be regulated, and also a requirement to regulate 25 additional substances every 3 years, which was physically impossible. As a result several MCLs were produced for substances that were unlikely to be present in significant amounts or nationally widespread or of significant concern. The compliance monitoring over the years has aptly demonstrated that, so there are actually more current regulations than needed to assure drinking water quality. The 1996 amendments required EPA to produce a Contaminant Candidate List (CCL) every 5 years (3 produced to date), make a determination every 3 years whether up to 5 meet the criteria to warrant possible regulation, and also to periodically generate lists of unregulated contaminants to be analyzed (UCMR) in a number of locations to determine occurrence and exposure from water. In my opinion the CCLs and UCMRs have generally not been critically prepared to isolate high probability substances of potential concern in drinking water. Although systematic processes have been utilized to produce them, there must be a serious flaw in the decision logic as has been demonstrated by their results.

The current regulations contain Maximum Contaminant Levels or Treatment Requirements for a broad spectrum of contaminants:

- **81 MCLs for Organics and Inorganics including:**
  - **27 pesticides and 9 DBPs (indicators)**
  - **21 Inorganics**
  - **24 VOCs and other synthetic organics**
- **Microbial: 6 Treatment requirements, Total Coliforms, E. Coli = all pathogens**
- **5 (2 group) Radionclide MCLs + Uranium = All radionuclides**
- **Lead and Copper corrosion control: Treatment Requirements**
- **Acrylamide and Epichlorohydrin: Treatment Technique as product quality.**

The coverage is actually far greater than the numbers imply. Essentially all microbial pathogens are covered by the MCL and treatment requirements. All radionuclides are covered by those rules, and treatment requirements such as surface water filtration and disinfection address numerous contaminants simultaneously. Disinfection byproducts

(DBP) indicator MCLs are intended to trigger techniques that result in across the board reductions of many unmeasured DBPs.

The existing regulations are very comprehensive as they cover almost all of the categories of potential drinking water contaminants including: microbial contamination in great detail, natural precursor products, inorganic chemicals, disinfection byproducts indicators, radionuclides, corrosion products, volatile and other synthetic organics, and pesticides. The potential for microbial contamination has been and will always be the greatest threat to health from drinking water. Although some outbreaks still occur, public water suppliers with Safe Drinking Water Act mandates have very successfully dealt with microbial threats, and no regulatory action should ever jeopardize our continued control over that threat.

As they should be, the regulations are designed to be protective, and they are produced with very conservative health assessments that are generally designed to overestimate potential risks. They utilize conservative default assumptions in the absence of detailed data, e.g. on margins of safety for toxicity, linear non threshold extrapolation models, and on occurrence and exposure and relative source contributions. I look at conservative risk assessments and regulations as a sort of insurance policy that we pay for to assure that there are not now and will not be meaningful risks from drinking water. However, conservative assumptions must be rational and carry mainstream scientific credibility, because, in a way they can convert risk assessors into pseudo regulators. The issue is ultimately how large should the safety margins be, and how much insurance cost is appropriate. There is a price, and over regulation can also have negative consequences, such as by unnecessarily increasing costs to consumers, and by limiting use of some beneficial technologies. Also, excessively dwelling on hypothetical and negligible risk concerns by regulators and the press also drives some people to mistrust their public drinking water supply and pay the extra (perhaps 1000x) cost of bottled water.

- **Examples of Some Pending Issues**

When producing a national regulation, EPA must, by law as well as common sense, determine that the contaminant is of national significance, is a risk to health, and also

demonstrate that the regulation will achieve meaningful health risk reduction. Here are several examples of current issues that are being debated and considered for regulation by EPA, including perchlorate, pharmaceuticals and nitrosamines. It is useful to put them into perspective relative to the context of the SDWA mandates.

- **Perchlorate**

Perchlorate in the environment, diet, and drinking water at low parts per billion levels, is potentially both of natural and anthropogenic origin. Perchlorate (as well as many other common anions) competes with iodine uptake by the thyroid and under some condition and dose could cause adverse consequences especially in infants. There has been much debate in the US, and some states have set standards or action levels at very low parts per billion (ppb) levels. EPA produced a guideline at 15 µg /L (15 ppb) in drinking water, and is now engaging in a regulatory development process. On the other hand in 2011, JECFA, the Joint Expert Committee on Food Additives, an independent multinational external toxicology/health science advisory committee of the UN Food and Agriculture Organization and World Health Organization released their analysis of the level of concern for perchlorate in Report 959. After reviewing the toxicology, epidemiological and clinical evidence, the NAS report from which EPA derived its current value, and dietary and water concentrations including much US data, they calculated a provisional maximum tolerable daily intake (PMTDI) of 10 µg/kg bw/day and drew the following conclusion:

> **"The estimated dietary exposures of 0.7 µg/kg bw/day (highest) and the 0.1 µg/kg bw/day (mean), including both food and drinking water, are well below the PMTDI. The committee considered that these estimated dietary exposures were not of health concern."**

- **Pharmaceuticals**

In recent years there have been reports of detections of several pharmaceuticals in some drinking waters mostly at parts per trillion levels. Pharmaceuticals can reach drinking water from upstream wastewater discharges and runoff from some animal feed lots. They are attenuated to some degree partly by the wastewater treatment process, environmental passage (biodegradation, decomposition and dilution), and drinking water treatment. Most of the entry of pharmaceuticals into wastewater comes from human excretion of

pharmaceuticals and metabolites after therapeutic uses; some fairly small portion derives from improper disposal of unused drugs. One week ago the World Health Organization issued a report that included contributions from a 10 member Working Group on Pharmaceuticals in Drinking Water, of which I was a member, and which reviewed much US and other data. WHO concluded:

> **"Trace quantities of pharmaceuticals in drinking water are very unlikely to pose risks to human health because of the substantial margin of exposure or margin of safety between the concentrations detected and the concentrations likely to evoke a pharmacological effect." (*i.e. the therapeutic dose*)**
>
> **"The current levels of exposure to pharmaceuticals in drinking water also suggest that the development of formal guideline levels for pharmaceuticals in the WHO *Guidelines for Drinking Water Quality* is unwarranted."**

Furthermore, WHO went on to say that routine monitoring and installation of specialized drinking water treatment was not deemed necessary.

- **Nitrosamines**

Nitrosamines are formed in some industrial processes, ubiquitous in foods, generated during cooking of proteins, and produced by humans endogenously from ingestion of precursor substances and oxynitrogen compounds. They are also found in some drinking waters at parts per trillion levels, especially those with upstream wastewater discharges, and also in some cases from disinfection with chloramine and from some polymers used as part of water treatment. Almost all nitrosamines are considered to be carcinogens. EPA has listed several nitrosamines in its Contaminant Candidate List (CCL 3) and in unregulated contaminant monitoring lists.

The regulatory challenge is that the portion of daily exposure to nitrosamines due to drinking water in those locations is likely less than 1% of all the other sources. The monitoring costs are substantial, and water treatment possibilities are not completely defined. Because the relative source contribution from drinking water sources is so minimal, a low Maximum Contaminant Level that would impact some number of those water supplies that contain some nitrosamine would have a negligible, if any, impact on risk and public health.

- **Unregulated Contaminants**

In theory, addressing unregulated contaminants in a formal way is a reasonable concept to determine which substances may warrant further assessment and possible regulation because of exposure from water and potential health risk. However, my sense is that EPA's approach to date has not been very efficient or effective. The process has been operating for more than 10 years and a huge amount of preparative and assessment work has been done, but there have not been any new regulations developed to date. In fact, that points to the obvious conclusion that there are not very many contaminants that demand national regulation to protect public health beyond those already on the books. On the other hand, I think EPA could have arrived at the same conclusion and identified perhaps a few candidates for regulatory consideration by a much more direct and efficient process. It should be said in EPA's defense that they were partly driven to that process by advice from a National Academy of Sciences report and from their statutory National Drinking Water Advisory Council.

- **Recommendations**

Overall public drinking water quality in the US is very good and the drinking water is safe, and undoubtedly safer than it has ever been since the introduction of centralized water supplies more than 200 years ago, and introduction of collected wastewater discharges. The potential for microbial contamination has and will always be the greatest threat. Public water suppliers with Safe Drinking Water Act mandates have very successfully dealt with microbial threats, and no action should ever jeopardize our continued control over that threat.

However, concerns are commonly raised because current and rapidly developing analytical technologies continually produce new detections at parts per billion, parts per trillion and even lower levels, and this trend will continue and expand, because analytical science becomes more sensitive. Those exposures are essentially always extremely unlikely, if not implausible, to have any meaningful risk. These amounts are many orders of magnitude below levels that can show a detectable effect from even high dose testing.

The number of substances in the water environment can be large, but the amounts are very small, and the likelihood for requiring national drinking water regulations to protect public health for many of them present at minute trace levels is not great. As a practical matter, it is simply physically and economically impossible, and also unnecessary to have a long list of regulated substances in drinking water. The resources and time required to produce regulations are great and the process is not responsive to the needs of water suppliers and public health officials to have access to a scientifically credible basis for rapid decision making when a substance is detected. That is precisely why the EPA Office of Drinking Water initiated the Health Advisory program in ~ 1980, and about 200 Health Advisories currently exist.

Risk assessments always utilize conservative default assumptions when detailed information is not available. Scientific knowledge progresses constantly and sometimes the risks may turn out to be less than previously estimated or assumed, so it is essential for credibility to always allow or require EPA to utilize the best mainstream scientific information when it is making regulatory decisions. It might be necessary to amend the "anti backsliding " provision in the SDWA to allow that to occur.

- **Suggested Drinking Water Act Implementation Strategy**

We have accumulated a significant history of drinking water composition and quality in the US and causes of contamination have decreased, so it would be logical and appropriate to utilize that knowledge to revise our approach to assuring the safety of public water supplies. Following is a suggested more efficient strategy to cope with the realities of safe drinking water provision and assurance of continued safe and safer drinking water.

1. Eliminate regulations that do not meet significant risk, national significance and meaningful risk reduction tests. Many regulations on the books have negligible occurrence near levels of concerns demonstrated by many years of compliance monitoring. Convert them into Health Advisories (see # 7). This might require legislation.

2. There needs to be application of rational and faster prescreening and prioritization methodologies such as the Threshold of Toxicological Concern (TTC) that has been used for years by the FDA and other organizations to screen food additives.

3. Add probably a small number of regulations that would meet the 1996 SDWA tests (national prevalence, health risk and meaningful risk reduction), and periodically update as needed.

4. When regulating, utilize individual MCLs, Treatment Technologies, or groupings as justified and technically appropriate. These have been traditionally utilized in implementation of the Safe Drinking Water Act, to date.

5. Always utilize the best available mainstream science when performing risk assessments and making regulatory decisions.

6. Emphasize addressing ageing distribution system infrastructure, because it is the likely greatest current and future drinking water health risk concern that requires correction.

7. Periodically carry out national surveys of source and finished drinking water composition so as to be up to date on what is present and might warrant regulation.

8. Greatly expand the number of peer reviewed Drinking Water Health Advisories (DWHAs) on substances that have been or would probably be in drinking waters. This is very cost effective and it would provide a compendium of substances in drinking water with authoritative guidance on health significance. It is responsive to the needs of water suppliers and public health officials for making rapid judgments in the event of a detection of a new substance.
e.g. DWHAs for most pesticides and pharmaceuticals can be readily produced from existing very robust registration and drug approval data bases.

9. EPA should provide guidance and assistance to states to help them develop local need regulations-health advisory assessments, technology performance and cost, and analytical methods.

10. EPA should facilitate applications of needed and new technologies developed by others, e.g. provide technical assistance to states and water suppliers by technology demonstrations, and via Technology Verification programs.

11. More aggressively use Clean Water Act, Toxic Substances Control Act, and Pesticide authorities to reduce contaminant introduction and burdens on Public Water Supplies.

12. Focus on preventing environmental and drinking water contamination. Industrial discharge controls have been successful. Manage pharmaceuticals by improved sewage treatment and controls on disposal, and pesticides by water basin management controls on applications.

13. Focus CCLs and UCMRs on most likely candidates rather than as shopping lists containing only a few candidates of likely concern.

14. Partner with states for sharing validated water monitoring data in a usable common electronic format so that information on national circumstances and trends is always readily available.

This proposed strategy is:

- **Comprehensive and forward looking, anticipatory and sustainable.**
- **Provides coverage of many more contaminants, essential benchmarks, and nationally consistent advice.**
- **Consistent with the SDWA mandate to protect public health.**
- **Much more efficient and cost effective for EPA, the states and water suppliers.**
- **Reduces wasteful compliance monitoring for public water suppliers**
- **Further reduces source contamination.**
- **Reduces uncertainties for water and state and local regulatory officials and the public.**
- **An opportunity to get ahead of the curve on interpretations of significance of trace contaminant detections using margins of safety and margins of exposure (MOE) to provide officials and the public a better perspective on DW quality and "risk" e.g. pharmaceuticals.**
- **It provides timely leadership and national consistency on water health related issues, (applications in Hazardous Waste Sites, Superfund…)**

Downsides—not many.

- **States might not always regulate their local need contaminants- EPA has supervisory oversight authorities under the SDWA implementation.**
- **State regulations will not always be uniform---they aren't always now.**

The bottom line is that this approach is responsive to the need, sustainable, credible, and efficient, and it provides more public health protection and much more bang for the buck!

Thank you for the opportunity to present these comments and suggestions.

Environment and Public Works Committee Hearing

July 12, 2011

Follow-up Questions from Senator James M. Inhofe and responses for written submission from Joseph Cotruvo

1. **Given the limited resources that utilities and EPA have when making investments in improving drinking water, where do you think the area of greatest need is?**

The greatest need is certainly not for new regulations that add costs with questionable or marginal benefits, if any. Currently, there is excellent regulatory coverage of significant contaminants and perhaps only a few additional might be appropriate in the near term, if they can be properly justified according to the requirements of the 1996 amendments to the Safe Drinking Water Act. The principal needs are for investment in infrastructure repair and replacement and especially in water distribution system infrastructure. Leaks, water loss or unaccounted-for water are major cost drains and sources of waste for water supplies and the environment, and of public resources. Recent information from the Singapore and Tokyo water systems indicates that due to their continued commitment, their unaccounted-for water is now on the order of 5% or less, which is much lower than in the recent past. By comparison Philadelphia estimates on the order of 30% unaccounted-for water and that is not unusual in the US. Pipe breaks in old systems also result in major disruptions and costly repairs. Contamination can enter leaking distribution piping during periods of low pressure. Infrastructure replacement needs to be accelerated, because it is cost effective to bring older water systems up to shorter average lifetimes. This would reduce water loss, pipe breaks and emergency repairs. Infrastructure upgrades are economically and environmentally beneficial and sustainable because they reduce waste of water and money.

2. **How would increased federal assistance in fixing and maintaining infrastructure provide meaningful opportunities for public health improvement? Would funding the SRF program be important in accomplishing this?**

Improvements in water treatment provide high quality water entering water distribution systems. The incidence of reported waterborne disease outbreaks has been reduced in the US, but the portion of distribution-related outbreaks and cases has been increasing. Much of this trend is due to legionellosis cases. Old and leaking distribution systems can result in recontamination or regrowth of some microorganisms, partly due to inability to maintain disinfectant residuals in the system. Old pipe is commonly cluttered with tubercle encrusted surfaces that harbor biofilms and microbes, and restrict the water flow. Better maintained and cleaner systems result in cleaner water reaching consumers. Federal assistance by revolving loan funds to be repaid at interest provide incentives for water suppliers to improve their

systems. This is cost effective by improving water safety, reducing leaks, water losses and pipe breaks, so that the economics of water production would improve for most supplies. It also is a source of labor intensive shovel-ready projects for job creation for the long term, because it requires a continuous commitment from the water supplier.

3. **California and Massachusetts recently set MCLs for perchlorate at 6 ppb and 2 ppb, respectively, and EPA may set an even lower level for drinking water. Based upon your experience, does reducing perchlorate to levels like these have any additional health benefit to the public?**

Perchlorate is present in the environment from both natural and anthropogenic origins. It is among the several anions that compete with uptake of iodine by the thyroid. In its unregulated contaminant monitoring rule data from 2001-2005 EPA found that 4.1% of the more than 3800 systems tested exceeded 4 µg/L (4 ppb). So, it is not a very widespread issue in drinking water. Perchlorate is not unique to drinking water; it is also present at parts per billion levels in numerous foods. EPA's 2009 interim guideline of 15 µg /L (15 parts per billion) in drinking water utilized a conservative 2005 recommendation from the National Academy of Sciences of 24 µg /day. This was based upon a non adverse NOEL (No Observed Effect Level) of 7 micrograms per kilogram of body weight per day, and reference dose of 0.7 micrograms per kilogram of body weight per day, that is "likely without appreciable risk of deleterious effects over a lifetime". The reference dose value is conservative because it was derived from a No Observed Effect Level (NOEL) rather than a NOAEL (No Observed **Adverse** Effect Level), and then an additional 10 fold uncertainty (safety) factor was applied. USFDA did not recommend changes in diet, and it did not recommend taking dietary supplements with additional iodine. EPA is now engaging in a regulatory development process and the outcome is unknown, but the implication is that EPA thinking is that the guideline is too high and should be reduced.

In 2004 the American Thyroid Association reported results from occupational studies indicating that "even long-term, intermittent high perchlorate exposure does not appear to alter thyroid function significantly, and it would also be expected that perchlorate in drinking water may not have a significant effect." A Chilian study (Tellez, 2004) of nonsmoking pregnant women determined that perchlorate in drinking water at levels as high as 114 µg/L (114 ppb) during pregnancy did not affect maternal thyroid status early in gestation or fetal thyroid stratus at birth.

The 2011 analysis of the Joint Expert Committee on Food Additives (JECFA), an independent multinational external toxicology/health science advisory committee of the United Nations Food and Agriculture Organization and World Health Organization came to a very different conclusion than EPA, CA or MA. In their report, they stated that none of the ecological studies

showed any relationship between perchlorate concentrations in drinking water and the incidence of thyroid diseases. After reviewing the toxicology, epidemiological and clinical evidence, including the NAS report from which EPA derived its current value, and dietary and water concentrations including much US data, JECFA calculated a provisional maximum tolerable daily intake (PMTDI) of 10 µg/kg bw/day, with a 10 fold uncertainty (safety) factor to cover high risk subgroups. This value is equivalent to 700 µg per day for a standard US 70 kg person, and 100 µg per day for a standard 10 kg infant/child. JECFA concluded that current daily exposures from food and drinking water were well below that PMTDI level so they were not of health concern.

Thus, the current EPA Guideline of 15 µg /L (15 ppb) in drinking water is already conservative and protective of health, and the much lower California and Massachusetts standards would not be expected to result in any benefits. According to the JECFA conclusions all of those values (EPA, CA, MA) would be extremely conservative. Thus, a low drinking water standard would not reduce risk or contribute to greater benefits to public health.

4. **EPA's Office of Inspector General has concluded that of the three common goitrogens (thiocyanate, nitrate and perchlorate), perchlorate amounts for less than 1 percent iodide uptake inhibition, while the other two chemicals account for more than 99% of uptake inhibition. What is the meaningful opportunity for public health risk reduction through the regulation of perchlorate, given that more than 99% of the effect is related to other chemicals?**

Numerous anions have some competition with iodide for transport into the thyroid, and anions are ubiquitous. Perchorate has high binding affinity, but the other anions are present in much greater amounts. Nitrate is frequently present in drinking waters and also in the diet both naturally, e.g. in vegetables like spinach, and as an additive in some processed foods. Thiocyanate is primarily a natural dietary component from several vegetables like broccoli and cabbage, and endogenously produced from precursors in the natural diet. Smokers have particularly high levels of thiocyanate in their systems. Much experimental toxicology has been conducted with rats, however, rats are much more sensitive to disturbances in thyroid function than humans, so rat data must be carefully interpreted and probably deserves uncertainty factors of less than one rather than the usual multiples of 10 that are common for animal to human extrapolation. Thus, human data are the best types of information for human risk assessments for these types of chemicals and endpoints. A scientifically valid analysis would not consider the anions individually, but evaluate aggregate effects from all sources to determine whether there is a risk that needs to be reduced and, if so, which strategy would be most effective and sensible, especially for smokers.

5. How do deficiencies in IRIS risk assessments affect EPA's ability to make regulatory decisions in the Drinking Water program?

IRIS has the role of providing objective, timely, comprehensive and authoritative risk assessments to support EPA's regulatory programs. To accomplish this it must produce scientifically credible assessments and in a timely manner. The draft chromium VI IRIS document is a recent example of a non scientifically credible IRIS assessment as demonstrated by the comments from the recent external peer review that EPA conducted. In order to be credible IRIS must represent mainstream scientific thinking and not be an organ for either internal or external fringe positions that could unreasonably constrain policy choices. Its work must undergo very detailed external broad spectrum independent peer reviews by qualified credible scientists, and then its conclusions must be practical and represent the weight of scientific evidence along with the alternative options and their strengths and weaknesses. IRIS should not wander into hypothetical risks that are very small, low probability and *de minimis*, of dubious validity, and which can never be verified. It must include the peer review results in its recommendations to the regulatory programs. The EPA regulatory programs usually have scientific staff, and the responsibility to produce beneficial and defensible regulations, so they should have the opportunity to not accept the IRIS conclusions for good cause, if they are not defensible.

With a few exceptions, drinking water is usually among the smallest contributors to human exposure to natural and anthropogenic environmental contaminants; diet is surely the greatest source of exposure and risk for most natural and synthetic substances. It could be argued that in some cases drinking water regulations may give excessive attention to drinking water and skew priorities when it is a negligible contributor to actual risks.

6. Other comments

A. The Safe Drinking Water Act as amended in 1996 has the elements needed to provide continuing protection of public health through safe drinking water. However, EPA's recent implementation strategy needs to be updated to become more efficient and provide the kind of comprehensive guidance that public water supplies need to cope with new potential concerns or questions that may arise. The existing regulatory list contains numerous superfluous standards that just add monitoring and administrative costs without commensurate benefits. The cost and time required to produce a regulation are very high, so only a few substances would warrant that expenditure. At the same time water suppliers and states and the public are left without benchmarks to address questions about significance or lack of significance of trace substances that are being detected by ever more sensitive analytical methods.

National standards should only be developed within the three determinants of the 1996 SDWA amendments: 1. National significance, 2. Risk to health, and 3. Meaningful risk reduction. Localized issues should be dealt with by states with technical assistance from EPA. The testimony contains a suggested 14 point comprehensive strategy that provides a starting point for EPA to update its approach to providing leadership for safe drinking water assurance and to be responsive to the current and future needs of all of the stakeholders. Its principal recommendation is a shift away from producing more costly and inefficient MCLs and standards to much greater emphasis on scientifically based and peer reviewed Health Advisory guidelines that can be much more comprehensive and timely and supportive of the roles of the states, and much less costly to produce.

B. The current Safe Drinking Water Act has an "antibacksliding" provision that makes it very difficult for EPA to raise an MCL if more current scientific data demonstrates that the actual risk from the substance in drinking water is less than what was thought when the original MCL was written. This utterly rejects the essentiality of good science in making regulatory decisions and requires that water suppliers make continued expenditures to meet a discredited standard. Any water supplier or state has the right to meet a lower standard if they choose to do so, and can justify it to their constituents, but it should not be required by federal law. This provision is a candidate for reconsideration when the congress next reviews the Safe Drinking Water Act.

C. Wastewater reuse for beneficial purposes is also worthy of much greater encouragement by the federal government. Wastewater has finally been recognized as a beneficial resource that should be maximally exploited, rather than treated and discarded. It is a source of nitrogen and phosphorous nutrients, biosolids for beneficial land application, oils and grease as potential biofuels, methane for energy production, and water for irrigation, as well as highly treated water for direct or indirect human consumption. Treatment technology of recycled water for potable use is similar to desalination of seawater and only about half the cost. It has been demonstrated at full scale that the current advanced wastewater treatment processes can produce water that is at least as high as and probably higher quality than good quality natural waters. Recycle programs for commodities like paper, plastic, and glass are usually difficult to justify economically because of the high cost of collection and transport. On the other hand wastewater recycling already has the collection and transport system (sewers) in place, so it is an ideal application. Wastewater recycling has the additional benefit of reducing discharges to the water environment, so it helps to clean our waterways.

Beneficial wastewater reuse is a phenomenon whose time has arrived. It is a win, win, win!

Senator BOXER. Your time has passed, and we need to move on. We thank you.

Dr. Steven Patierno, we are glad that you are here. And let me give your background to the folks. Executive Director of the George Washington Cancer Institute. We are very happy you are here. And is also Vivian Gil here with you?

Mr. PATIERNO. I am sorry?

Senator BOXER. Is Vivian Gil here with you?

Mr. PATIERNO. No. Vivian Gil is a historical donor to the George Washington University endowing a professorship, which I hold.

Senator BOXER. Aha, that is what I was going to say. It says, this is so funny, it says Vivian Gil Distinguished Professor of Oncology, so I thought she was a distinguished professor.

[Laughter.]

Senator BOXER. But we have you, and you are the distinguished professor. So we are thrilled that you are here. Go ahead.

STATEMENT OF STEVEN R. PATIERNO, PH.D., EXECUTIVE DIRECTOR, GEORGE WASHINGTON UNIVERSITY CANCER CENTER

Mr. PATIERNO. Before I start, I would like to also quickly apologize for the length of my comments. I only found I was invited to attend this on Friday and just didn't have time to hone this down to exactly 5 minutes, as some of my colleagues have.

Senator BOXER. We don't have a choice, because we need to go on. So please, go ahead. Do your best.

Mr. PATIERNO. Madam Chairman, Ranking Member Inhofe, and members of the Committee, thank you for this opportunity to address you today. As you have heard, my name is Steve Patierno, I am the Director of the GW Cancer Institute. I am also a professor of pharmacology and physiology in the School of Medicine and a professor of environmental and occupational health in the School of Public Health.

I have been conducting research for over 31 years on hexavalent chromium, 23 of those funded by the National Institute of Environmental Health sciences and the National Cancer Institute. I have published many papers, served on many review committees. I would also like to disclose that although I have never worked for, consulted directly for any company associated with any chromium-related industry, over the course of 30 years of working in chromium, I have on seven occasions served as an expert for the defense in chromium litigation.

A recent 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 in 31 U.S. cities. Most of the cities with the highest of these low levels of ambient chromium–6 have little or no proximity to any chromium-related industry, indicating that these levels likely constitute a natural background, results that are neither new nor unexpected. The report and the associated media coverage, which was purposeful in referring to chromium–6 as the carcinogenic Erin Brockovich chemical, has caused unnecessary fear and alarm, as these levels constitute no health risks to humans.

It should be stated that there is a vast literature on occupational and industrial exposures to high doses of chromium compounds for long periods of time, a large literature on animal studies and defined systems. Valid conclusions are only drawn when complementary data come together and fundamental principles and practices of pharmacology and toxicology must be factored into an accurate analysis.

Taken together, the consistent message is that only long-term high dose exposures to chromate dusts, as encountered in the chromate production pigment and plating industry have been associated with human cancer, and only for cancers of the respiratory tract. The same studies also showed there was no consistent association with any increased risk of other cancers. This is attested to by every major government international agency review ever written.

All three major areas of risk related study, epidemiology, animal studies and cell culture provide clear evidence for very high threshold levels for both toxicity and carcinogenicity. And in fact, epidemiologic studies can identify no effect levels despite long-term occupational exposures that are factored over a 45 year work history. Likewise, published meta-analyses, investigated gastrointestinal tract cancers, and all of this analysis shows no significant risk in malignancy for any GI site.

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

Evidence for a very high threshold level for chromium carcinogenicity is found in the recent NTP animal studies where rodents were administered very high concentrations of chromium–6 in the drinking water continuously for 2 years. Tumors were only observed at the two highest doses, and they were so high the water was unpalatable to rodents. And they resulted in long-term chronic tissue damage. There is a figure in my comments. I would ask you to note the sharp high-dose threshold for toxicity and that no toxicological effects were observed, even at doses that are nearly five orders of magnitude, 100,000 times higher than the average tap water concentration reported in the EWP report.

Despite Hollywood depictions, chromium–6 is not potent as either a toxic or a carcinogen. Enormous quantities of chromium are needed to evoke any kind of toxicity in humans or animals. This is because humans have a many-tiered, innate mechanism of protection against chemical toxicities of any sort, including chromium. It is critical to understand that chromium compounds exist in two forms, a trivalent form, which is an essential element required for human physiology, and a hexavalent form. Hexavalent chromium is very, very rapidly and completely reduced to trivalent chromium by a number of protective mechanisms and fluids in the human body. And recent studies have shown that this conversion takes place in less than a minute.

If it does reach a cell, there are intra-cellular mechanisms of protection that prevent it from directly interacting with DNA. So it is not correct to assume that if a vanishingly small amount of hexavalent chromium reaches a cell, it automatically becomes carcinogenic.

[The prepared statement of Mr. Patierno follows:]

Testimony of Dr. Steven R. Patierno, Ph.D.
Executive Director, George Washington University Cancer Institute

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).

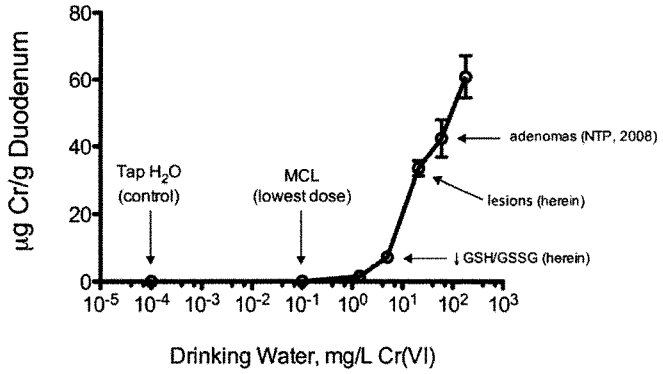
Furthermore, all three major areas of risk-related study, (epidemiology, animal and cell culture) provide clear evidence for a very high threshold levels for both toxicity and carcinogenesis. For example, in an analysis of occupational risk in a cohort of more than 2300 workers in the chromate production 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/m^3-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/m^3-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/m^3-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.

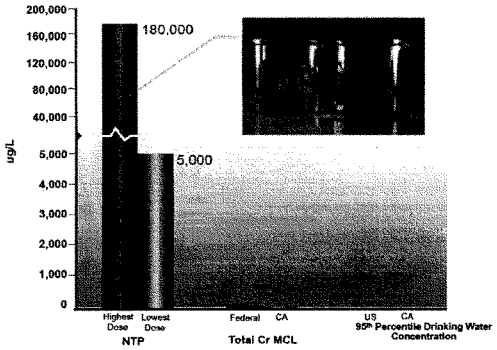
Likewise, a published Meta-Analysis investigated Gastrointestinal Tract cancers (oral, esophageal, stomach, small intestine, colon and rectal) in all epidemiology studies of Cr(VI) exposed workers published after 1950 (Gatto et al. 2010), including 32 studies from various industries wherein airborne 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 dihydrafte) 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 conduted 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, is it 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 fluxuation 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 at, 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 is 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/m^3 but can range up to 500 ng/m^3. 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 CrIII, as a potential hazard (see preceding comments). Except for those few citations it is almost universally accepted that CrIII 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 posses 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 ooncentration 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 profieration, (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 know 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 a either a mutagen or a carcinogen and in fact there is strong evidence that it functions as an 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 uM 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 uM 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 uM) 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 extracelluar 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 chonic 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 dis-similar 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 mutagenecity, 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 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 Glascow 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 Massachussets 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 carcinogencity 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 know 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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November 3, 2011

Jonathon Aronchick
Senate Committee on Environment and Public Works
410 Dirkson Senate Office Building,
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Dear Mr. Aronchick,

Below you will find my attempt to answer the questions posed to me by Senator Inhofe. As I did at my public testimony, and as I did both before and at the EPA Peer Review of the Draft IRIS Risk Assessment for Hexavalent Chromium, I disclose that over the course of a long career of research in chromium toxicology and carcinogenesis I have occasionally served, and am currently serving, as an expert witness in chromium-related litigation (<10 cases in 30 years). This is normal activity for experts in the applied science of toxicology.

Because one of the Senator's questions refers to an ongoing, multi-institutional research study (funded by the American Chemistry Council and coordinated by ToxStrategies) on the MOA and pharmacokinetics of hexavalent chromium exposure in rodents, I also disclose that the George Washington University (my employer) was awarded a small grant from ToxStrategies to conduct a small part of this very large multi-center study. I am not the Principle Investigator (PI) of that grant. Although the PI of that grant is a professional colleague of mine he is an independent faculty member and he does not work for me or report to me in any way.

1. EPA has released a Draft IRIS Risk Assessment for hexavalent chromium. I understand that you were invited by EPA to participate on its peer review panel for this draft assessment and that the peer review comments were submitted to EPA. What was the gist of your comments to EPA on its Draft IRIS Assessment? In the draft Assessment, EPA uses a linear approach to develop its unit risk value. Did you agree with that approach?

I was a member of the peer review panel for the draft assessment and my detailed comments are publically available. This gist of my comments to EPA is that an objective analysis of all lines of scientific inquiry into chromium carcinogenesis (occupational epidemiology, in vivo animal studies, and in vitro studies on cultured cells) point to a clear non-linear dose response curve for toxicity and tumor formation. There are clear no effect levels of exposure and even those no effect levels are very high exposure levels. Toxicity and tumor formation are only observed after very high levels of exposure for long periods of time and those high doses are associated with chronic tissue damage and subsequent chronic tissue regeneration. In rodent studies little or no tissue accumulation of total chromium can even be observed until mice are continuously exposed (with no option) to at least 14mg/L (ppm) of concentrated sodium dichromate in drinking water. Even under these chronic high dose conditions there is little or no change in even the most sensitive indices of exposure until the chronic exposure reaches at least 60 mg/L (ppm), and tumors are not observed unless the mice are exposed,

continuously for their lifetime, to 170-520 mg/L (ppm). At least one reason for this is that the vast majority of the hexavalent chromium taken in by these animals is reduced to trivalent chromium which is an essential nutrient for humans. In my opinion, and in the opinion of a strong majority of the Review Panel, it is inappropriate to linearly extrapolate from uniquely high dose effects, to speculative low dose potential, when there are clear no effect levels in between.

2. We understand that recent mode of action and pharmacokinetic research findings were presented at the peer review panel meeting. What was your impression of this research? Should EPA include these new data in its revised Assessment? Are these data pivotal to our understanding about hexavalent chromium in drinking water?

I assume that this question is referencing the large multi-institutional study that is funded by the ACC and coordinated by ToxStrategies. This study is being conducted at six or more independent laboratories including several unaffiliated academic institutions. Several papers from this study have already been published in the peer-reviewed scientific literature and apparently more are on the way. The stated goal of this research is to conduct MOA studies using the identical animal model and doses used in the NTP bioassay and to address gaps left unaddressed by the NTP assay. Given that these studies are being conducted in independent laboratories by independent investigators these data should be included in the EPA's revised Assessment. My recollection is that it was unanimously agreed upon by the Review Panel that the EPA should wait for and utilize this data. Judging from the published papers that I have seen so far, these data strongly reinforce the concept of the non-linear dose-dependence that I described above, so in that specific sense they are important but perhaps not pivotal (we already knew this from the NTP study. However, these studies are also providing very important insights into the MOA of hexavalent chromium under high dose exposure conditions.

3. If the mode of action and pharmacokinetic research findings presented at the peer review panel meeting are not included in EPA's risk assessment, will the risk assessment provide an accurate picture of the current state of the science on hexavalent chromium oral exposure?

As I conveyed in my public comments, the current Draft Assessment does not adequately consider even the existing data (prior to the current MOA study organized by ToxStrategies). All lines of scientific inquiry point to clear no effect levels, even at high levels of exposure. Independent of the ongoing MOA study, a strong majority of the Review Panel urged the EPA to reconsider its conclusion of a mutagenic mechanism of action. At least four of the members of the Panel, including myself, have spent many years studying the interactions between hexavalent chromium and DNA, and we each have contributed to the peer-reviewed literature on what we presumed was mutagenesis by hexavalent chromium. Most of us have gradually moved towards the opinion that although Cr(VI) can be forced to show what we presumed to be mutagenic activity in heavily contrived experimental systems, this "mutagenesis" only occurred at very high doses that killed the vast majority of exposed cells, suggesting that it was more likely that we were just selecting for chance survivors. Additional studies, wherein the presumably mutated genes were subjected to DNA sequencing, no consistent mutations or patterns of mutations in the base sequence of the genes could be found. Taking this exceedingly weak pseudo-mutagenesis together with the non-linear in

vivo dose response, and the fact that tumors were only observed at exceedingly high dose which also caused chronic tissue damage, most of us have shifted our thinking towards chronic tissue damage and its associated cell regeneration as a more probable MOA for high doses. At the low end of these high doses (which are still very high) there is no MOA because there are no measurable effects.

4. Is the National Toxicology Program (NTP) study consistent with other scientific literature on the carcinogenic threshold exposure for hexavalent chromium?

See answer to Question #3 above.

5. In February, this Committee heard from EPA that they believed the NTP study argued for more stringent drinking water controls for hexavalent chromium. Based on your review of current literature, do you think this study calls into question the need for additional regulation of drinking water to protect people from getting cancer from hexavalent chromium?

The NTP study merely confirmed what most experts already knew. Mammals, including humans, possess enormous capacity to convert hexavalent chromium to trivalent chromium which is non-toxic and non-carcinogenic and is actually an essential nutrient. Only a very small proportion of chromium exposure is ever actually absorbed into the body and even if a small amount hexavalent escapes conversion at the point of entry or in the stomach, the blood and the other tissues possess additional enormous capacity to convert Cr(VI) to Cr(III) and bind it up in manner that prevents Cr(VI) from arriving at tissues or getting absorbed into cells as Cr(VI). At exceedingly high doses this protective capacity can be overwhelmed and both toxicity and carcinogenesis can ensue, but even then there are additional layers of intracellular protection against toxicity, mutagenesis and carcinogenesis. The NTP study merely confirmed that exceedingly high concentrations of hexavalent chromate, administered in rodents' drinking water with no option for a lifetime, can produce toxicity and carcinogenesis. At lower doses in the same study (which are still exceedingly high) there is a fairly dramatic threshold below which no toxicity or carcinogenesis can be observed. This strongly supports a non-linear dose response and the existence of a high-dose threshold, below which there is no effect.

6. Did any member of the risk assessment review panel believe EPA should rely on the journal articles from Critical Reviews in Toxicology you mention in your testimony? Is a risk assessment that relies on such articles one that can stand up to scientific review?

This was not discussed during the Review Panel so I have no way of knowing whether any panel member relied on those articles. My concerns regarding those articles can be found in my Comments on the draft Assessment.

7. Is there anything else you would like to add for the record?

Not at this time.

Steven R. Patierno, Ph.D.
Director, The GW Cancer Institute
Vivian Gill Distinguished Professor of Oncology

Professor of Pharmacology and Physiology, Genetics and Urology
The GW School of Medicine and Health Sciences
Professor of Environmental and Occupational Health, and Global Health
The GW School of Public Health and Health Services
Founding Director, Molecular and Cellular Oncology Program

Senator BOXER. Thank you very much, sir.

We are going to move to our final witness, Dr. Jeffrey Griffiths, Associate Professor, Department of Public Health and Community Medicine at Tufts.

STATEMENT OF JEFFREY K. GRIFFITHS, MD, MPH AND TM, CHAIR, DRINKING WATER COMMITTEE, SCIENCE ADVISORY BOARD OF UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, PROFESSOR OF PUBLIC HEALTH AND OF MEDICINE, TUFTS UNIVERSITY SCHOOL OF MEDICINE

Dr. GRIFFITHS. Thank you, Chairman Boxer and Ranking Member Senator Inhofe, members of the Committee. Good morning. I really appreciate this opportunity to testify.

My name is Jeff Griffiths, I am actually a professor now at Tufts University. And I am Chair of the Science Advisory Board's Drinking Water Panel which advises the U.S. EPA. I am trained as a physician, internal medicine, pediatrics, infectious diseases, epidemiology. Relevant to this, I have been involved in regulation for about 15 years of drinking water contaminants. In fact, I was a member of both the National Academy of Sciences panel which recommended some changes in the way the EPA looks at unregulated contaminants, as well as the National Drinking Water Advisory Committee when it also then chewed on that report and then made some suggestions to the EPA.

So I would just like to comment a bit on some of the ways in which I see there needs to be a sea change in the way these things are looked at. One of the things that is really important to acknowledge is there is a sea of new chemicals out there that we are finding. We are just finding more and more and more of them as detection capacity increases. Unfortunately, health effects data is really hard to get. It is expensive, it is time-consuming, et cetera. So we really only have health information on a small number of contaminants.

So this, I think, means that the number of chemicals has essentially outstripped our capacity to get health information very easily.

The second point that I would like to make along that line is of course that the health effects differ, depending on who you are. If you are a baby or pregnant mother or something like that, it is going to differ depending on what population you are part of.

The next point that I would like to make is the need for transparency and a good public process. It forms a good, robust scientific basis for decisionmaking, because that means that things are out in the open. And also some confidence on the part of the public in terms of accepting the conclusions that are made about these compounds. And I would just comment that the 1998 contaminant list was considered quite murky and relied on expert opinion. You are going to hear a diversity of expert opinions about these contaminants. So I think the transparency is really important.

I just wanted to go through the perchlorate story a little bit, because it seems to me that kind of illustrates these points. First off, we didn't really know that perchlorate was in the water until detection capacity improved. That happened in the 1990's. So then suddenly people were finding perchlorate everywhere. So that

means that monitoring and actually, good monitoring is really critical.

The second point that I would make along that line of the perchlorate story is that it has been known for a while that perchlorate affects thyroid function. But because of the interest in perchlorate there were new studies done, there is new animal data. And some of that animal data, for example, suggests that perchlorate acts on thyroid function in a different way than we had thought and at lower levels.

So that is consistent, in fact, with data that was accumulated by the CDC in doing a study of nearly 3,000 people where they found that women living in Atlanta and throughout the Country had less thyroid hormone in their system if they had more perchlorate in their system. So there seemed to be some kind of an inverse relationship. So if you are exposed to perchlorate, you may have lower thyroid levels.

And then I think that illustrates the point that once something gets on a list, it means there is going to be more science done about this thing. So it has to make it onto the list.

The last point that I would like to make in this story is that if we didn't know anything about a specific sensitive population with perchlorate, we might have less concern. That is because perchlorate is concentrated in breast milk. So when a mother is nursing, she may be delivering high levels of perchlorate. And sort of perversely also less iodine than the baby needs. If you didn't look at that particular population, you wouldn't know this.

And I have some data in here about that in my remarks. But the key thing is that by looking at a susceptible population, suddenly there is this new information that goes, ah, OK, so this is a group that is more susceptible not only inherently but because of the way mom delivers breast milk. So I think it is a really critical thing that there be data collected from susceptible populations.

I want to comment that there were many people such as myself who were surprised that perchlorate was not identified for regulation in 2008. It was a controversial decision. And many people thought there was a lack of transparency around that. And that goes to the original point that I made, that transparency is really crucial in this.

Drinking water contaminants are really high on the public discourse, and I think there are four things that have to be done. We have to look at groups of compounds for regulation. We need to devote sufficient resources, so that we can monitor these compounds. We have to look at health effects, both in the general population and in the susceptible populations. And last, the whole process has to be transparent, because this will maximize scientific credibility and public confidence.

Thanks very much.

[The prepared statement of Dr. Griffiths follows:]

Oral Testimony of Jeffrey K. Griffiths, MD MPH&TM

Chair, Drinking Water Committee, Science Advisory Board of the US EPA

Professor of Public Health and of Medicine, Tufts University School of Medicine

Senate Committee on Environment and Public Works

Oversight Hearing on the Environmental Protection Agency's Implementation of the Safe Drinking Water Act's Unregulated Drinking Water Contaminants Program

July 12, 2011

Chairman Boxer, ranking Member Senator Inhofe, and Members of the Committee, good morning. Thank you for this opportunity to testify today before the Committee on Environment and Public Works regarding the implementation of the Safe Drinking Water Act's unregulated drinking water contaminants (UDWC) program.

I am Jeffrey K. Griffiths, MD MPH&TM, a Professor of Public Health and of Medicine at Tufts University School of Medicine, and Chair of the Drinking Water Committee of the US EPA's Science Advisory Board. [My professional training has been as a pediatrician, internist, and infectious diseases epidemiologist. I was the Director of the public health program at Tufts University for five years, and have studied waterborne diseases for over twenty years. I have been involved in the national regulation of drinking water contaminants for approximately 15 years, and served on the National Drinking Water Advisory Council for over a decade]. I was a member of both the 2001 National Academy of Sciences' National Research panel, and the 2004 National Drinking Water Advisory Council committee, which recommended a significant sea change in the way the US EPA it assesses unregulated drinking water contaminants. We recommended that contaminants be selected for regulation using a systematic, scientifically sound, and transparent process. Please allow me to elaborate as this relates to today's hearing.

An increasing number of contaminants and our capacity to understand their health effects

In order to implement the UDWC program, both water occurrence and health impact information is needed. Many challenges exist. Factually, ever more contaminants are being found in water as our detection capacities improve. However health effects information can be expensive and time consuming to acquire, and is currently available for only a small number of contaminants. The

number of known unregulated contaminants has now outstripped our capacity to analyze them all on an individual basis using traditional approaches. Furthermore, health effects differ for people at different life stages; what may be safe for one group may not be safe for another. These challenges must be acknowledged and overcome - but cannot serve as an excuse for inaction.

Transparency, public participation, and confidence in the process

A transparent and public process promotes a robust scientific basis for decision making, and confidence with acceptance by the public. The 1998 candidate contaminant list determination was quite murky and extensively relied on expert opinion. As well we know, experts can differ in their opinions. Implementation of the UDWC program should be transparent, welcome stakeholders and the public, and be mindful of social equity issues.

The perchlorate story illustrates the importance of these points.

Perchlorate was unexpectedly discovered in ground water when water testing improved in the 1990s, and was found to be present in groundwater throughout the country. This demonstrates the benefits of continuously improving the monitoring of our water supply.

In the 1990s, perchlorate was already known to interfere with the thyroid gland's uptake of iodine. Since then, new animal data[1] suggests that perchlorate's adverse effects on the thyroid are found at unexpectedly low levels of exposure. Similarly, work by the Centers for Diseases Control (CDC) has shown that thyroid hormone levels were significantly lower in women who were exposed to low levels of perchlorate which had not been thought to affect thyroid function.[2]

This data, and data from many other studies, suggests that perchlorate is of public health concern. It demonstrates the value of appropriately focusing resources so that new health information can be generated which may dispel, or increase, concerns about a contaminant.

Perchlorate has now been found to be concentrated in breast milk. It may replace iodine which is essential for babies. Breast-feeding mothers may provide their infants with inadequate iodine and perversely feed them excessive perchlorate. A 2007 study [3] concluded that 90% of the babies of women who drank water containing perchlorate at the 2006 preliminary groundwater remediation goal set by the EPA of 24.5 micrograms per liter would receive a daily dose nearly three times the maximum amount established for infants. [This information was used by Massachusetts to set a drinking water standard of 2 micrograms per liter,[4] currently the most stringent standard in the United States]. The point is that breast fed babies get all of their nutrition from Mom. They are fed breast milk which concentrates perchlorate, and so their mothers have to drink low levels of perchlorate to protect their infants from perchlorate enriched milk. This information about a specific susceptible population provides a key insight into the potential health effects of perchlorate which would have been missed otherwise.

It was surprising to many, including me, when in the face of this information, the EPA did not target perchlorate for regulation in 2008. This decision was controversial and used by many as an example of a lack of transparency in the UDWC evaluation process. Indeed, the fact that not one new chemical compound has been identified for regulation since the adoptions of the 1996 Safe Drinking Water Amendments suggests that implementation has been hampered by a lack of

scientific resources, a lack of data, or inadequate transparency. Many of the decisions to not regulate contaminants have been driven by a lack of information.

In sum,

Drinking water contaminants continue to be high on the burner of public discourse. In addition to perchlorate, unregulated compounds such as chromium VI, volatile organic compounds (VOCs) such as trichloroethylene and its chemical relatives, and a variety of agricultural and pharmaceutical compounds are of public health concern. The EPA has the opportunity to re-invigorate the implementation of the unregulated drinking water implementation process by:

(1) analyzing groups of similar compounds for regulation when appropriate;
(2) devoting sufficient resources so that worrisome compounds are adequately monitored on a national basis;
(3) studying health effects in both the general population and in sensitive subpopulations; and
(4) using an open and transparent process, which will maximize scientific credibility and build public confidence.

Thank you for this opportunity to comment and I welcome any questions.

Cited publications

[1] McLanahan ED, Andersen ME, Campbell JL, Fisher JW. Competitive inhibition of thyroidal update of dietary iodide by perchlorate does not describe perturbations in rat serum total T4 and TSH. Environ Health Perspect 2009; 117:731-738.

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[3] Ginsberg GL, Hattis DB, Zoeller RT, Rice DC. Evaluation of the US EPA/OSWER preliminary remediation goal for perchlorate in groundwater: focus on exposure to nursing infants. Environ Health Perspect 2997 115:361-369.

[4] Zewdie T, Smith CM, Hutcheson M, Rowan West C. 2010 Basis of the Massachusetts Reference Dose and Drinking Water Standard for Perchlorate. Environ Health Perspect 2010 118:41-48.

Senator BOXER. Thank you.

Senator Inhofe has asked if he can ask his questions first.

Senator INHOFE. Mine will be very, very brief. I do have a lot of questions I want to ask for the record that I will be submitting.

Dr. Patierno, I know that you have done extensive research into chromium–6, with particular emphasis on the carcinogenic and mutagenic efforts. As you know, my constituents in Norman, Oklahoma are very much concerned about this. I just wanted to get your expert opinion. First of all, is chromium–6 mutagenic? And second, are my constituents at health risk from drinking water at the low levels of chromium? I am talking about Norman, Oklahoma now, because I think you are aware of what is going on there.

Mr. PATIERNO. Yes. Thank you for the question, Senator Inhofe. The question of whether hexavalent chromium is mutagenic has become a very interesting question. Those of us who work in DNA damage and carcinogenesis have known for years that in carefully contrived experimental conditions, we can force virtually any chemical to become carcinogenic, or excuse me, mutagenic. We have been concerned for a long time that in order to detect mutagenicity after exposure to chromium, one has to use enormous concentrations that actually kill the vast number of cells or tissue cells that get exposed.

We have always been concerned about that. Recent studies have gone back in, actually sequenced the DNA sequence of the genes that we thought had been mutagenized, and found that the DNA sequence is still intact. And that has led to a current understanding, or an emerging understanding, that despite the fact that you can force chromium–6 to get into cells at very high doses and damage DNA, or bind to DNA, it appears that the mode of action is that we are killing cells and selecting for survivors.

So that has raised very significant questions that came up 2 months ago in the review panel for the toxicological review of chromium, and extensive discussion was made about that extensive question.

Senator INHOFE. Are they at risk?

Mr. PATIERNO. Are they at risk at the levels in tap water?

Senator INHOFE. Yes.

Mr. PATIERNO. Absolutely not.

Senator INHOFE. OK, that is what I wanted them to hear.

The last question I have is for Dr. Cotruvo. I appreciate your statement and your emphasis on fixing and maintaining infrastructure and reducing leaks and contamination. I was telling the Chairman here that I went through this experience when I became mayor of Tulsa. We had a deteriorated infrastructure.

Do you think that the funding, the SRF program, the revolving fund program, would be important in accomplishing some of this infrastructure correction that we need?

Mr. COTRUVO. Certainly, the financial demands are huge. Somehow we built all this without a lot of Federal support over the last 200 years. But it has really come to the point where there is significant infusion that is needed to get these systems back up. And probably Federal funds are going to be necessary.

Senator INHOFE. But revolving funds is what I was referring to.

Mr. COTRUVO. Yes.

Senator INHOFE. OK, thank you very much. Thank you, Madam Chairman.

Senator BOXER. Thank you.

Dr. Patierno, I am struck by your confidence that chromium–6 is safe, and I am going to press you on that. First of all, you argue that too much was given to the animals. Isn't this what we do in science? Because of their life span, isn't that a normal test, that you give a lot to the animal so that you can see what is going on?

Mr. PATIERNO. Yes, that is a standard procedure. I am not arguing that too much was given. What I am arguing is that so much was given that it was, it overwhelmed all possible protective mechanisms. And even at very, very, very high doses, not effect was observed until the two very highest doses, which went hand in glove with extensive toxicity to the tissue.

Senator BOXER. But isn't that being conservative, in your approach? Isn't it the point? I understand that you have testified as a defense witness, isn't that correct, on chromium–6 cases?

Mr. PATIERNO. I have on occasion.

Senator BOXER. Yes, you have. You made money doing that?

Mr. PATIERNO. I have.

Senator BOXER. Yes, you have. I don't make money on any side of this thing. I am trying to protect the public, OK? So when you tell me too much was given, I want to be conservative about this.

So Dr. Goldman, I think it gets to your point, and Dr. Griffiths as well, I would like to ask you to comment on the cumulative effect. You made the strong point, and I think Dr. Griffiths as well, what we are dealing with here, we are looking at something where if a little child is exposed, let's say even in utero and continues to be exposed to these contaminants, it is a cumulative impact, is it not, Dr. Goldman?

Dr. GOLDMAN. Yes, it is. It is a cumulative impact. It is also the impact of exposures over a lifetime. And I should say in the case of hexavalent chromium that this has been reviewed by expert bodies, like the International Agency for Research on Cancer. And they do conclude that it is a risky substance. They do not conclude that they can absolutely assure safety at the levels of exposure in the population.

Senator BOXER. Dr. Griffiths?

Dr. GRIFFITHS. Thank you. The issue of mixtures of chemicals is really a big one. Because we have a way in science of doing this kind of dancing on the head of a pin where we have the chemical or no chemical. But the truth is we live and we drink water with lots of different things in it. And we have exposures not only through water but air and food and so forth. And we really don't have good tools for figuring that stuff out.

So I think that we in fact do have to develop new tools. But also, what information we do have suggests that exposure to multiple chemicals, even at low levels, can be risky. So I think it is important to understand that sometimes it is not just one thing. It is like smoking and asbestos exposure. Your rates of lung cancer are really high if you both smoke and have exposure to asbestos. But they are much lower if you only do one.

Mr. PATIERNO. May I comment?

Senator BOXER. Dr. Patierno, yes, I am going to let you have the floor in a minute. I am going to ask you this question. The National Toxicology Program, which is part of the U.S. Department of Health and Human Services, found that chromium–6 in drinking water caused cancer in the studies of animals. And you have taken issue with how much they got, I don't know exactly what you said, you said, I don't think they got too much, but it is how much—I was confused on what you were talking about.

And then the EPA's draft assessment on chromium–6 has found that this toxic heavy metal is "likely to be carcinogenic to humans when consumed in water." So you take issue with this?

Mr. PATIERNO. Madam Chairman, it is not just that I take issue. All nine members of the external review panel for the draft toxicological assessment of hexavalent chromium disagree with that assumption. It has to be qualified by dose. And the assessments that are done by international agencies on the carcinogenicity of chromium deal specifically with high level occupational exposures. And they even cite in the literature these are exposures as encountered in the chromate production pigment and plating industries. Because of the unique chemistry and biology of chromium, of hexavalent chromium, and the capability of our bodies to reduce hexavalent chromium very quickly to trivalent chromium, which is completely non-toxic, and in fact, is an essential element, make it unique among other chemicals and make it so that it is not scientifically sound to do a linear dose extrapolation at high doses.

Senator BOXER. OK, OK, I get it. You don't agree with it. I get it. Now, is there anything you agree with? We have standards for 88 different chemicals, I am sure you are aware of this, over the years. Do you believe that these are safe and shouldn't have had standards associated with them?

Mr. PATIERNO. I have not looked into any of the others. I was only invited here today to speak on——

Senator BOXER. I know. But you are making a statement, you were a witness for the defense, I am questioning your objectivity. So I am trying to find out if you just feel that way on—I assume you think it is fine to limit the amount of chromium in the water, total chromium, is that correct?

Mr. PATIERNO. Total chromium?

Senator BOXER. Yes, total chromium, which is in fact regulated and has been for quite some time.

Mr. PATIERNO. I am not sure I am following specifically your question.

Senator BOXER. I am asking a question. There is 88—you are an expert—there are 88 chemicals that are regulated under the law where they have maximum contaminant levels. This piece of paper tells me that chromium is regulated. Are you aware of that?

Mr. PATIERNO. Yes, I am.

Senator BOXER. OK. Do you support that?

Mr. PATIERNO. I do support that chromium needs to be regulated based on the occupational exposures that are so well characterized in the literature.

Senator BOXER. This is drinking water. This isn't occupational. This is the Safe Drinking Water law of the land. Do you agree that there should be a limit on chromium in drinking water?

Mr. PATIERNO. Madam Chairman, the question for me is not whether there should be. There of course should be. The question is at what level.

Senator BOXER. OK, how about arsenic? Do you think we should regulate arsenic in drinking water? Yes or no?

Mr. PATIERNO. Madam Chairman, I am here today to speak on hexavalent chromium.

Senator BOXER. No, you are not really. You are not really. You are here because we are having an overview of the Safe Drinking Water program. And we are not asking you just on chromium. What about benzene? What about chlorine? Do you support that?

Mr. PATIERNO. Any chemical that has potential toxicological impact needs to be evaluated and regulated. But each chemical needs to be evaluated based on the science supporting that specific chemical, the mechanisms of action of that chemical, the defense mechanisms that the body has against particular chemicals. All of that needs to be taken into consideration.

One could argue using a different set of criteria that something as common and innocuous as acetaminophen shouldn't be on the market because two might control a headache but 50 could dissolve your liver and create a lethal situation. So it is all about dose and form and duration. And then the basic principles of toxicology which argue in this case that human beings have very, very, very strong defense mechanisms, particularly against low levels of hexavalent chromium.

Senator BOXER. Well, good luck with that theory, because you ought to talk to some people who had exposure who don't feel that way. But you are a scientist, and I am saying to you, we have 88 different chemicals that are regulated here. And if people came with your attitude and appeared in court in favor of the defense, we wouldn't get anywhere. And we have a problem here, and that is why we called you together. Because since 1998, we haven't regulated anything.

And I think what you hear from the panel basically is, we may not have to regulate a lot of things. We agree that maybe, as Dr. Cotruvo said, existent in, and echoed by Dr. Griffith, they may be appearing in very small, minute quantities. And that is why we have to look at the risk factors, who is exposed to these, the cumulative impact, as Dr. Goldman suggested, and not just say, we appeared for the defense. That is just not going to get us anywhere, when money gets involved in the equation.

We need to have pure science. And that is what I am for, pure science.

Now, we will be very interested to see where the EPA takes it on perchlorate. And I could tell you that you just talked to my constituent here on perchlorate, when you have 800, what is it, 800 parts per billion?

Mr. ARAIZA. Yes.

Senator BOXER. Instead of six?

Mr. ARAIZA. Yes.

Senator BOXER. And you smell that, as I have, and you see it, it is scary. It is scary.

Mr. ARAIZA. Yes.

Senator BOXER. And we know the Safe Drinking Water Act is going to protect the most vulnerable. We are not talking about protecting a 250 pound man who is 6 foot 5 here. That is what we just to do. But guess what? A Boxer amendment a very long time ago did something your clients didn't like, Dr. Patierno. We said, you have to protect the most vulnerable child. That was my amendment, God bless it, it passed. And so, no, we are going to be conservative when we set these standards, and we are going to look to see how we can protect the most vulnerable.

But I want to thank the panel. You have all been very, very helpful. We are going to move on and see what happens with these rules on chromium–6, on perchlorate. We are going to see what happens when the EPA adopts many of the recommendations made by the GAO, because clearly, no one can tell me that we are any safer today when we haven't even regulated one thing since 1998. It just doesn't add up, when you look at the rates of cancer out there. Let's just get real here.

Especially I say to the doctors, we have cancer in people younger and younger. We have serious problems, we are spending billions of dollars, we don't have a cure. And I will be darned if I am going to sit here and allow us to say that this isn't important. This is very important and we are going to do our work.

So we look forward to seeing the EPA's response. I am very encouraged by today's hearing, because I think the GAO's very good work is going to lead us to more science, hopefully less politics, more transparency and more action. So thank you very much, all of you, for being here today. We stand adjourned.

[Whereupon, at 12 p.m., the committee was adjourned.]

[Additional material submitted for the record follows.]

STATEMENT OF HON. JEFF SESSIONS, U.S. SENATOR
FROM THE STATE OF ALABAMA

Good morning Mr. Trimble and Dr. Perciasepe. Thank you for coming before our committee today.

We are here today to consider EPA's implementation of the Safe Drinking Water Act, which is designed to protect our nation's drinking water supplies from harmful contamination. As the Ranking Member of the Subcommittee on Water & Wildlife, I am interested in learning more about this topic.

Under this law, EPA currently regulates more than 90 drinking water contaminants—things like cryptosporidium, chlorine, E-coli, and arsenic, just to name a few. These are contaminants that we all want to ensure do not damage our drinking water. EPA is required by the Safe Drinking Water Act to periodically review existing standards as well as to consider whether new standards should be issued.

I am aware that GAO has issued a report expressing an opinion that EPA should improve the way it decides whether to regulate additional contaminants. I am interested in learning more about that report.

In addition, there are 4 issues, in particular, that I think we need to be sure to keep in mind.

First, the Role of the States. The Safe Drinking Water Act, like many of our Federal environmental laws, is based on a concept known as "cooperative federalism." EPA oversees the program, but the States have a critically important role in implementing and administering the program.

In Alabama, our State environmental management agency works closely with more than 700 water systems in the State to ensure safe drinking water for our state's 4 million citizens. The State enforces compliance with the drinking water standards. There are monthly, quarterly, and annual monitoring and reporting requirements to ensure compliance. By and large, our State has very good drinking water, and I believe we have an effective drinking water program.

We cannot lose focus of the fact that what we do here, at the Federal level, results in mandates on the states. Those mandates are, in large measure, usually unfunded.

So, as we explore the possibility of greater regulation, we need to ensure there is close consultation with the states, as well as with the water systems themselves.

Second, the Impact of New Standards on Water Systems and Water Users. When a new Federal MCL (maximum contaminant level) is issued, the water systems have to comply with that new standard. That becomes a new cost imposed on those who pay for the water. Rural water systems, in particular, often carry the heaviest burden when trying to comply with these standards. I think we need to be as flexible and reasonable as possible to ensure that excessive costs are not imposed on our rural communities.

Third, the Role of Science—does the science justify new standards and the costs of those standards. If there are costs to be imposed, we need to make sure that the underlying decision to create any new standard is correct—based on the best available science. I think we need to know more about EPA's methodology for establishing drinking water standards, and in particular, EPA's "IRIS" system.

Fourth, our Nation's serious infrastructure needs. As Senator Inhofe has articulated, we have an aging infrastructure in this Nation. Our water infrastructure is, in many cases, being used well beyond intended lifespans. I am interested in learning more about the impact of our aging infrastructure on our ability to ensure safe drinking water.

Thank you.

Peer Review Workshop for EPA's Draft Toxicological Review of Hexavalent Chromium

Reviewer Post-Meeting Comments

July 6, 2011

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(A) Oral Reference Dose (RfD) for Hexavalent Chromium **49**

A1. A two-year drinking water study of sodium dichromate dihydrate in rats and mice (NTP, 2008) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study. 49

A2. Diffuse epithelial hyperplasia in the duodenum of female mice was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect. 51

A3. Benchmark dose (BMD) modeling was applied to the incidence of diffuse epithelial hyperplasia in the duodenum of female mice to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of diffuse epithelial hyperplasia) scientifically supported and clearly described? 53

A4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale. 54

(B) Carcinogenicity of Hexavalent Chromium **56**

B1. Under EPA's 2005*Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgrd.html), hexavalent chromium is *likely to be carcinogenic to humans* by the oral route of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described? 56

B2. A mutagenic mode of carcinogenic action by all routes of exposure is proposed as the primary mode of action for hexavalent chromium. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for hexavalent chromium that may support an alternative primary mode of action. 59

B3. A two-year drinking water study in rats and mice (NTP, 2008) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantification is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered. 72

B4. The incidence of adenomas and carcinomas combined in the small intestine of male mice from the NTP (2008) two-year drinking water study were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment. 73

B5. The oral slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk of tumors of the small intestine in male mice). Has the modeling been appropriately conducted and clearly described? 75

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Responses to Charge Questions

General Charge Questions

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazard?

Reviewer	Comments
Byczkowski	Logical - yes; clear - mostly; concise - no, as there is a very large body of information covered by this Toxicological Review. While the scientific evidence of both noncancer and cancer hazards of oral exposures to Cr^{+6} has been appropriately reviewed and synthesized, the conclusions and numerical derivation of toxicity values are mostly based on strict (default) interpretation and literal application of U.S EPA guidelines, rather than on the current scientific understanding of the mode of action of Cr^{+6}.
Hamilton	In general, the report is a concise presentation of the vast primary literature for chromium toxicology and carcinogenicity and the writing is generally clear. However, there are specific sections with deficiencies as noted below in detail, and many other sections and specific comments that are not logical. In many cases a statement in one section is either not a logical extension of the data presented, or is in opposition to a statement elsewhere. Overall, the greatest concern is with the logic regarding the choice of a mode of action (MOA), which is the basis for many of the subsequent assumptions that are made, the default values that are chosen, and risk assessment modeling that follows from these choices. In this reviewer's strong opinion – and in the consensus opinion of the external reviewers who are experts in this area and who discussed this at the May 12, 2011 meeting – Cr(VI) is highly unlikely to act via a mutagenic mode of action in vivo. Rather, a careful review of existing information, as well as emerging studies all strongly indicate that the likely MOA involves a threshold mechanism that supports both the physiological uptake-reduction model of DeFlora and the cellular uptake-reduction model of Wetterhahn that were previously proposed. The current EPA draft document concludes that chromium(VI) acts via a mutagenic MOA by all routes of exposure, a conclusion that is illogical given the current state of knowledge of chromium biology and toxicology as already presented in this draft report, and also based on the recently emerging data from a series of 90-day rodent MOA studies sponsored by the American Chemistry Council (ACC). This is the most important and central point since the choice of a mutagenic MOA then drives all other considerations in this document. Specific areas of concern are outlined by chapter and section below in detail in a combined response to G1. and G2. **Chapter 1** EPA should include more definitive information about the literature it reviewed that contributed to this draft report. It currently states that the relevant literature was reviewed through September 2010 but the external reviewers all noted at the May 12 meeting a number of gaps in the literature being cited in the current draft. In addition, it would be helpful to know how many studies the EPA identified as being part of the chromium literature, how many it reviewed, how many it set aside or did not review, what criteria

	were used to include or exclude a study, etc. For example, a statement such as: *"The EPA identified 26,839 peer-reviewed scientific publications in PubMed from 1950 through September 2010 using the keyword 'chromium.' Of these, 9,456 were determined to be relevant to the current draft based on the criteria of covering aspects of chromium biology, toxicology, environmental chemistry and epidemiology."* **Chapter 2** On page 14 the draft states that,*"Natural occurrence of hexavalent chromium is rare ..."* This statement should be qualified since geochemical surveys by the U.S. Geological Survey and others have indicated that there are background levels of naturally occurring Cr(VI) in most groundwater, and that these levels are typically in the range of 2-5 ppb but can be up to 3-5 times higher than that in certain areas. Likewise recent reports of Cr(VI) levels in soil and house dust indicate that there are natural sources, or at least sources that cannot be identified as being specifically anthropogenic, that contribute to the background levels and background exposures of people throughout the U.S. A more detailed literature review and discussion regarding background levels of Cr(VI) in soil, air and water, including more up-to-date information on such levels, should be included in this chapter to provide context for subsequent discussions regarding human exposure through drinking water. Keep in mind that, until recently, analytical methods to detect total chromium and to speciate chromium(VI) accurately were difficult, with higher detection levels than many natural sources contain. Further, speciation of chromium(VI) at very low levels has been very problematic until recently, so much of the older environmental data are inaccurate or had non-detect values where reanalysis has revealed widespread chromium(VI) background levels in the environment. This is of importance not only for understanding the potential contribution of these background levels to total chromium exposure but also in setting practical regulatory levels. Clearly it is of little value for the EPA to calculate a maximum contaminant level (MCL) or public health goal (PHG) that is lower that typical background levels since this would be virtually impossible to achieve by most public drinking water systems with limited resources, and because such background levels are unlikely to represent a significant health risk (see discussion below). **Chapter 3** This is an important chapter that would greatly benefit from reorganization and from synthesis of information. A number of studies are described here sequentially, and for several of these the draft document speculates on possible mechanisms. However, the order of presentation is not logical, and there is little in the way of more global synthesis of results and the conclusions that can be drawn – or those areas that remain controversial or poorly understood – while there are several areas where a statement in one section is contradicted by a statement elsewhere. Because the toxicokinetics of chromium is so central to its biology and toxicology, this is a critical chapter for its overall evaluation and therefore this chapter should be revised. Regarding organization, Section 3.3 is extremely important for understanding the studies described throughout the remainder of the chapter, providing context for extracellular versus intracellular reductive metabolism. It is suggested that this section should be moved to just before or immediately after the current Section 3.1. Likewise, Section 3.6 on Cr(III) and its nutritional benefit and essentiality is extremely important for

	understanding how the body normally absorbs, distributes and excretes chromium. From the standpoint of human environmental exposures, Cr(VI) is primarily, but not exclusively an anthropogenically derived form of chromium that is principally encountered and used in occupational settings, but humans and all other life forms have been dealing, both physiologically and biochemically, with Cr(III) and reduction of background levels of Cr(VI) for the entire history of life on Earth. It is recommended that Section 3.6 should be moved to the beginning of this chapter and greatly expanded. The current section on this important topic is extremely short, superficial, and inaccurately presents this subject as an area of controversy in the field. Cr(III) nutritional biochemistry has been extensively studied over the past fifty-plus years with a large and robust literature. Only two toxicologists are cited as the sources of the "current debate" about this, whose individual views have been well aired in their review articles but which represent views that are generally considered to be well outside the mainstream of toxicology and nutritional biochemistry. Citing their views so prominently in this short section does not balance well with the wealth of studies and numerous other investigators over decades who have concluded that there is a beneficial role of Cr(III) in human and animal nutrition and have demonstrated an underlying biochemistry and physiology that supports this role. This section should be greatly expanded and treated in a more balanced fashion since it sets the stage for understanding how Cr(III) is treated by the body in all aspects of toxicokinetics, and therefore also provides valuable insight into Cr(VI) toxicokinetics that largely explains much of the experimental literature on chromium disposition in intact animals and humans as discussed in more detail below. See for example reviews by W Mertz (J Nutr 1993, 123:626-633; Nutr Rev 1995, 53:179-185), RA Anderson (Reg Tox Pharm 1997, 26:S35-41), HC Lukaski (Ann Rev Nutr 1999, 19:279-302) and JB Vincent (J Am College Nutr 1999, 18:6-12) for summaries of this earlier literature. Some of this information is alluded to – for example, the end of the primary paragraph on page 44 beginning with *"Aitio et al. (1988) developed ..."*, which represents an extremely important body of literature on Cr(III) toxicokinetics and which should be moved forward with section 3.6 and greatly expanded by citing other relevant literature. There are dozens of studies of Cr(III) uptake and kinetics. The current chapter leaves the impression that Cr(III) gastrointestinal (GI) uptake is uniformly low, and that chromium is not normally found above background levels in urine, therefore if one administers Cr(VI) and sees a dose-dependent increase in blood, urinary or tissue levels it is evidence of Cr(VI) uptake as Cr(VI). But there are a number of uptake studies of chromium picolinate and other natural and man-made Cr(III) complexes, as well as Cr(III) uptake in chromium-sufficient versus chromium-deficient diets, that defy this simple interpretation. For example, one study described on page 45 by Kerger et al. (1997) reported that *"ingestion of chromium picolinate resulted in significantly elevated urine concentrations such that participants routinely exceeded background."* Similar elevations have been reported for chromium picolinate in the nutritional literature. Likewise, other studies have shown an inverse correlation between Cr(III) levels in diet and the uptake, distribution, tissue storage and excretion of Cr(III), indicating that the body tightly regulates Cr(III) kinetics to maintain a steady-state body burden and availability of the nutritionally active form of Cr(III), and can actively take up Cr(III) from the GI when the body senses that it is deficient, or decrease uptake and increase excretion when internal stores are sufficient or exceeded.

	A related and key concept that is alluded to in this section but never addressed directly is the behavior of chromium in serum and red blood cells (RBC). It has been well established, beginning with RBC labeling studies going back to the 1950's in which RBC are incubated with Cr(VI) ex vivo, that Cr(VI) is readily taken up by RBC, rapidly reduced, and in the process forms highly stable chromium adducts on hemoglobin and other macromolecules which are very long lived, essentially remaining intact for the lifetime of the cell. In this way the half-life of RBC in humans and experimental animals was established (with human RBCs having a half-life of ca. 110-120 days) and this tool has also been used to look at RBC turnover. This is alluded to on page 27, where it states that *"The partitioning of hexavalent chromium from plasma into erythrocytes is significant. It has been used as a biomonitoring endpoint ... and is responsible for the observed residence time of chromium in whole blood ..."* Conversely, in the human and animal studies that were described in Chapter 3, it has been consistently shown that when Cr(VI) is administered orally or by gavage, there is a transient increase in both serum and RBC levels which rapidly return to baseline. Thus, it is highly unlikely that the chromium in the blood of these animals and humans was Cr(VI), or else the RBC would have been stably labeled. It was noted on page 45, for example, in describing the human studies of Paustenbach et al. (1996) where there was oral exposure to Cr(VI) that *"both plasma and RBC chromium concentrations returned rapidly to background levels within a few days, again suggesting that concentrations of 10 mg Cr(VI)/L or less in drinking water of humans appears to be completely reduced to Cr(III) prior to systemic distribution."* This is an extremely important experimental observation in humans -- and one of the most important statements in this draft document -- that directly addresses the issue of whether Cr(VI) is taken up by the human GI as Cr(VI) and whether it survives as Cr(VI) in the circulation. Similar results were seen in the Sutherland et al. (2000) rat study in which the blood kinetics, and lack of chromium increases in brain or other distal tissues argued strongly that it was Cr(III) rather than Cr(VI) that was taken up by the gut. Were this not the case, they should have observed stable chromium labeling of the RBC and elevated chromium levels in distal tissues. But they reported the opposite, and this also strongly indicates in this key rodent study that Cr(VI) failed to survive as Cr(VI) in crossing into the bloodstream from a GI exposure. And elsewhere in Chapter 3, similar results are reported that would lead to the same conclusion, yet the text alludes to transient RBC uptake as possible evidence that Cr(VI) is being taken up from the GI tract as Cr(VI). This is a highly flawed, illogical argument that appears throughout this document. Likewise it is now well known that there are specific uptake, transport and storage mechanisms for nutritionally active Cr(III) that must be taken into account in any measurements of chromium in the blood or other tissues. In fact, none of the studies presented in Chapter 3 provide any direct evidence that any Cr(VI escapes the GI tract as Cr(VI) except perhaps where normal gastric reduction is bypassed or the bolus doses are so large as to completely overwhelm the reductive capacity of the gut, which is likely what occurred in the NTP (2008) study. This should be more clearly described in this chapter, perhaps with a new section prior to current section 3.5 that presents the theoretical PBPK model since this model also critically relies on how one interprets the in vivo data.

	Specific edits suggested below are based on these concerns (changes underlined): • Page 24, Line 24 – "*...for the chromium administered as hexavalent ...*" • Page 25, Line 7 – "*...of the chromium administered as hexavalent ...*" • Page 26, Line 3 – "*.... Generally, absorbed chromium is ...*" • Page 26, Line 9 – "*... toxicokinetics of chromium, ...*" • Page 26, Line 16 – "*... comparing administration of Cr(III) ...*" • Page 30, Line 2 – "*... bioavailability of chromium administered as ...*" • Page 35, Lines 7-11 – two sentences beginning with "*The reason for the higher ...*" This is highly speculative and should be deleted. This could all be Cr(III) rather than Cr(VI) using the arguments above. • Page 36, Lines 25-28 – end of sentence beginning with "*... indicating that a portion of the Cr(VI) escaped extracellular reduction ...*" This is also highly speculative and is actually counter to the data presented, which clearly show transient blood levels that are indicative of Cr(III) distribution, not Cr(VI), as more appropriately alluded to in Lines 32-34 where is says "*Brain, ovarian, and whole-blood concentrations were below detection limits in all exposed groups. The lack of concentrations in whole-blood was attributed to rapid delivery of Cr to tissues and clearance of plasma Cr.*" The lack of stable blood chromium clearly indicates that it could not have been absorbed as Cr(VI) or else the RBC would have shown significant and stable elevations. • Page 38, Line 13 – "*... did not alter GI uptake appreciably at these concentrations.*" • Page 41, Line 1 – "*Chromium is capable of crossing the placenta.*" • Page 41, Line 15-17 – "*Absorption and elimination of chromium was evaluated .... Following ingestion by human volunteers of either trivalent or hexavalent chromium in single or multiple drinking water doses.*" • Page 42, Lines 23-26 – This statement beginning with "*Because the Cr(VI) increases ...*" is incorrect. Increases in RBC chromium that are stable over time, with the same half-life as the RBC, would be indicative of uptake of chromium as Cr(VI) by the RBC, but if the increase in RBC chromium is transient, as in this case, then it cannot be due to Cr(VI) in the blood but is most likely a Cr(III) complex that is carried by the RBC but not covalently bound. Evidence for this is cited elsewhere in this chapter (see middle of page 44 and top of page 46, for example) and in the broader Cr(III) literature. The draft report is illogical since it currently argues this issue both ways depending on the study being discussed. • Page 42, Lines 30-35 – These two sentences beginning with "*The higher bioavailability ...*" are highly speculative and well outside the boundaries of the actual data presented and discussed above. If the report is going to speculate, it should pull all this into a separate section and synthesize it across all the studies that were cited.

	Otherwise delete these speculations since they do not have a factual foundation. • Page 49, last line – " … *chromium administered as Cr(VI) distributes* …" • Page 58, Lines 2-3 – " … *greater percentage of chromium administered as Cr(VI) than Cr(III) is absorbed.*" Delete sentence reading "*This implies that some Cr(VI) escaped reduction* …" since this is not implied by the actual experimental data based on the above arguments. Taken together, these studies, as argued above, lead one to only one logical conclusion: Cr(VI) is not taken up by the gut as Cr(VI), nor does it survive as Cr(VI) in the systemic circulation in these studies. It is only by greatly exceeding the normal doses and reductive capacity of the gut that one can see any signs of Cr(VI) surviving to reach other tissues. Therefore there is a clear biochemical barrier, a threshold, to Cr(VI) uptake and systemic exposure under normal physiological conditions. This must clearly be taken into account in any analysis of MOA and resulting risk assessment for this toxicant. **Chapter 4** Liaoning Province, China studies – At the beginning of this chapter (Pages 68-76) the EPA presents an extensive review and discussion of the various reports related to the study of populations in China near a site of chromium-contaminated drinking water from a nearby industry. Depending on the authors and methods used – particularly certain assumptions regarding age adjustment, use of an urban area as a control population, and exposure estimates -- the reports of this data set either find no statistical association between stomach cancer incidence or a modest elevation. The other epidemiology studies cited in this chapter report no statistical correlation between drinking water chromium exposure and cancer incidence. It is of considerable concern to this reviewer that the EPA has chosen in some places to highlight this one positive report and elevate it to the level of their major recommendations, such as on Page 239 of Chapter 6 (Major Conclusions) where they state that there is "*evidence of an association between oral exposure to hexavalent chromium and stomach cancer in humans.*" Yet elsewhere, they state, appropriately, that "*this risk has not been established in other populations exposed to drinking water contaminated with hexavalent chromium. The epidemiology data are not sufficient to establish a causal association between exposure to hexavalent chromium by ingestion and cancer.*" (Page 201, Lines 20-23; and similar language on Page 205, Lines 33-36) It is inappropriate to "cherry-pick" a single study – indeed, a single treatment among three treatments of the same study – if this is going to influence their major recommendations regarding the human carcinogenicity of Cr(VI) via ingestion. Many of my other comments and edits relate to this concern, as well as previous concerns regarding the toxicokinetics of Cr(VI) via ingestion and the misinterpretation of those results with respect to mode of action and carcinogenic potential in animals and humans as detailed below. This reviewer feels strongly that the Liaoning Province study and its various treatments should be set aside, since the initial study (first reported in 1987) is highly flawed by today's epidemiology standards, there is a lack of information that would allow others to reevaluate this study in the manner that would most directly address the potential correlation, and the three subsequent treatments by original authors Zhang and Li (1997),

	by Beaumont et al. (2008) and by Kerger et al. (2009) -- each of which makes slightly different assumptions in order to fill critical data gaps -- reached different conclusions. It is also important to point out that this population was exposed not just to extremely high levels of Cr(VI) but also to industrial effluent which contained high levels of a number of other chemicals of concern including sulfates, acids and other toxicants. Thus, even if an association was found, it is not possible to attribute this to Cr(VI) per se and could be the result of either other contaminants or a statistical anomaly based on the high rates of stomach cancer in China which are diet- and province-related. For these reasons this study and its series of treatments cannot be the basis for risk characterization or risk assessment, and should be set aside. Section 4.4 also requires extensive revision and expansion. The first paragraph reports only briefly on the extensive literature examining occupational exposures and cancer, principally lung cancer. This section, and this literature, should receive a much more extensive treatment since these studies are our best data regarding human exposures to Cr(VI). It should be noted that in addition to inhaling extremely high levels of Cr(VI) prior to the advent of industrial hygiene practices in the 1960's and 1970's (levels as high as several mg chromium(VI) per cubic meter) these workers had extensive dermal exposures, and also had extensive ingestion exposure since it is well known that individuals exposed to high levels of dust swallow a large fraction of what they inhale either through direct deposition in the mouth, nose and throat, and via mucocilliary clearance of the pulmonary system. Thus, these occupational epidemiology studies are extremely important in understanding the toxicology of chromium(VI). Several key points will be made below citing a few critical studies, but the EPA should more thoroughly review this entire literature. The U.S EPA's current risk assessment for chromium(VI) via inhalation is based principally on the studies by Mancuso of older worker populations through the 1960's prior to the advent of modern industrial hygiene practices. Similar risks were observed in other occupational studies of workers from the 1940's through 1970's who were exposed to much higher levels of chromium than are encountered in occupational settings today, such as those by RB Hayes et al. (Intl J Epi 1979, 8:365-374; Am J Indust Med 1989, 16:127-133), JM Davies (Br J Indust Med 1984, 41:158-168), T Sorahan et al. (Br J Indust Med 1987, 44:250-258), and R Kishi et al. (Am J Indust Med 1987, 11:67-74). Subsequent epidemiology studies of workers exposed to lower levels of chromium since the 1960's have not only provided estimates of risk at these high doses, but have also provided important information as to lower occupational levels at which <u>no increases in lung cancer or other health effects were observed</u>. These more modern studies have also taken into account other factors, such as accounting for cigarette smoking and other confounding variables, whereas many of the older studies did not control for these important confounders (see for example, KD Rosenman, Am J Indust Med 1996, 29:491-500). It should be noted that, where it was reported, virtually all the lung cancer cases in these occupational studies occurred in smokers (see for example Pastides et al., Am J Indust Med 1994, 25:663-675; T-C Aw, Reg Tox Pharm 1997, 26:S8-12). These studies not only provide better estimates of the actual health risks attributable to occupational chromium exposure, but also an estimate of a practical threshold below which we would predict either no effects, or risk of effects that are so low that it cannot readily be detected

	even in large populations of exposed people. Gibb et al. performed a follow-up study (Am J Indust Med 2000, 38:115-126) of a worker population in Baltimore MD that had previously been studied by Hayes et al. (Int J Epi 1979, 8:365-374) in examining the relationship between chromium(VI) exposure and cancer incidence. In the Gibb study, the workers were stratified according to different levels of cumulative exposure to chromium, allowing a more detailed examination of the potential dose-response. Cumulative exposure was expressed as $\mu g/m^3$-years (1,000 ng/m^3-years), integrating both the chromium level and the total time of exposure at that level. This is similar to smoking data that express cumulative dose as "pack-years" and is based on the observation that the risk of a 40 pack-year smoker who used 1 pack per day for 40 years is similar to that of a 40 pack-year smoker who used 2 packs per day for 20 years. In the Gibb study, the lowest quartile of workers had exposure to chromium between 0 and 1.5 $\mu g/m^3$-years (1,500 ng/m^3-years). This group had an observed/expected lung cancer ratio of 0.96, i.e., it was slightly less than expected from the comparison population (the general population of Maryland) that had no occupational chromium exposure. Pastides et al. examined a group of chromate production company workers in North Carolina (cited above), focusing on the possible differences in risk between cohorts of workers who were exposed to chromium under the older conditions and processes of the 1940's through 1960's and those who began work after 1971 in a modernized factory in which both the chemical process and the exposure levels to chromium had been modified. They found a slightly increased risk of lung cancer, proportional to exposure, in the older cohort working under the higher dose exposure conditions, as had been reported previously in other studies. However, the workers in the modernized factory had no excess of lung cancer, all cancers, heart disease, or all causes of death over an 18 year period. Personal monitors for the workers indicated that the chromium(VI) levels were all below 50,000 ng/m^3, and most were below 25,000 ng/m^3, with the majority in the range of 500-10,000 ng/m^3. Average duration of employment was 9.5 years, such that cumulative dose would have averaged 4,750-95,000 ng/m^3-years. Dividing the workers into two groups of exposure, i.e., those working less than 10 years versus those working more than 10 years, indicated no difference in mortality, further suggesting that these workers had no significant increase in cancer or other health risks from either the higher or lower chromium exposures. Similarly, Aw reported (cited above) that workers in the more modernized plants who were occupationally exposed to chromium since the 1960's showed no increase in disease risk, as was also noted by S Langard et al. (Br J Indust Med 1990, 47:14-19). WJ Blot et al. performed a large and comprehensive study (J Occup Environ Med 2000, 42:194-199) of a group of 51,899 workers of the Pacific Gas & Electric (PG&E) Company. A sub-set of 3,796 these workers had been exposed occupationally to chromium(VI), either as gas generator workers or trainees at the Kettleman CA station which used chromium as a rust inhibitor in cooling tower water at PG&E natural gas transfer stations from the 1950's through the 1980's. Examination of these workers for specific cancers, all cancers, specific non-cancer diseases, and all diseases indicated no increased incidence in any adverse health outcomes in relation to chromium exposure. In fact, the total cancer and lung cancer standardized mortality ratios (SMRs,

observed/expected ratios) were 0.64 and 0.81, and 0.55 and 0.57, respectively, for these two groups of chromium-exposed workers, which was less than those of the overall PG&E worker group and substantially less than those of the general California population against which they were compared. SMRs for all causes of death were also low (0.79 and 0.68, respectively). Likewise, JD Boice et al. performed a large and comprehensive study (Occup Environ Med 1999, 56:581-597) of a group of 77,965 workers at an aircraft manufacturing plant in California. A sub-set of 3,634 of these workers were exposed to chromates and other chemicals as part of airplane production for a total of 88,224 person-years of exposure and a mean of 24 working years per person of exposure. The SMR for total cancers was 0.93, and the SMR for lung cancer was 1.02. As with the Blot study, there was no association of any adverse cancer or non-cancer health outcome with chromium exposure in this group, nor did the overall worker population have an increase in overall or specific mortalities as compared to the general population despite exposure to a number of occupationally related chemicals.

Taken together, these occupational studies indicate that, although previous historical exposure conditions were associated with a modest risk of lung and respiratory cancer (average of 2- to 4- fold increased lifetime risk, as compared, for example, to a 10- to 20-fold increased risk for cigarette smokers), more recent occupational exposures at or below the current regulatory limits indicate that these represent levels that do not elevate cancer risk even for lifetime occupational exposures. Moreover, the previous exposures of concern in workers from the 1930's through the 1960's were at levels that typically exceeded 1,000,000 ng/m^3 and also involved exposure to the most carcinogenic forms of chromates, i.e., the insoluble or slightly soluble forms such as lead chromate, zinc chromate and calcium chromate. The newer lifetime occupational exposure limits -- at which no increase in cancer risk or other health effects has been observed – represent daily exposures that are hundreds to thousands of times higher than would occur in an environmental setting or via U.S. drinking water. There are two other major conclusions that can be drawn from these occupational exposure studies. First, although dermal exposure to chromium(VI) was extensive – particularly prior to the advent of industrial hygiene practices in the 1970's – there is no evidence for increased risk of skin cancer, even in workers where the chromate levels were high enough to burn "chrome holes" in their skin or nasal septum. These chrome holes healed and were not associated with increased skin cancer risk in these workers. This is relevant to the very high doses of chromium(VI) used in the NTP studies and a possible MOA. Second, taken together these occupational studies do not demonstrate an increased risk of GI cancers or other internal cancers, despite the fact that these workers swallowed a significant fraction of the dusts they were exposed to in the air. These data were recently summarized in a meta-analysis published in 2010 (NM Gatto et al., Cancer Epi 2010, 34:388-399).

Other specific edits and comments (changes underlined):

- Page 200, Lines 1-2 – Delete the phrase *"... and evidence of an association between oral exposure to hexavalent chromium and stomach cancer in humans"* which is based on a single study and EPA's selective treatment of this result as discussed above.
- Page 200, Lines 18-20 – *"This study found evidence <u>of a modestly increased incidence of stomach cancer mortality (OR 1.69, CI 1.12-2.44)</u> from 1970 to 1978 ...."*

	• Page 201, Lines 20-23 – "... *was reported in a re-examination of a single study in JinZhou ..*" • Page 209, last section, Bioavailability – This section is highly flawed in logic and presentation as discussed under Chapter 3. Specific edits are as follows: o Page, 209, last two lines – "*Quantitative studies of GI absorption of chromium administered as hexavalent chromium have estimated ...*" o Page 210, first line – Please note that hexavalent chromium was not measured, and without exception has never been measured systemically as Cr(VI) following GI absorption. It is an assumption that increased chromium uptake to the blood represents Cr(VI) uptake, but it could also represent other forms as discussed in Chapter 3 above. We know that certain forms of Cr(III) are much more readily taken up than others, and it is therefore possible, and perhaps quite likely, that reduction of Cr(VI) in the gut in the presence of organic molecules in the GI lumen leads to formation of complexes that are much more bioavailable than inorganic Cr(III) that is typically found in food, water and soil. You could also modify this phrase as a separate sentence to read *"This may indicate that not all hexavalent chromium is reduced by the gastric juices of the stomach, or that reduction of Cr(VI) in this environment leads to formation of organic chromium complexes that are more readily absorbed than inorganic Cr(III)."* o Page 210, Lines 7-12 - Delete the sentence beginning *"Thus, at oral doses within human exposure ranges ..."* as per the argument above or modify to include alternative interpretations as suggested above. o Page 210, Lines 28-30 – End of sentence beginning with *"... and uptake of hexavalent chromium into the tissues ... "* delete this phrase or modify it as per the argument above. o Page 210, last line – Add a sentence after the last sentence on this page reading *"However, none of those studies speciated the chromium that was absorbed systemically, and so the form(s) of chromium in the blood and other tissues is unknown following increased absorption of chromium following ingestion of hexavalent chromium. Therefore, it is not known whether chromium reaches the blood or distal tissues as chromium(VI) at doses relevant to human exposures. The lack of long-term labeling of RBCs by chromium in the animal and human studies argues that little, if any, chromium is absorbed as chromium(VI) under these exposure scenarios."* o Page 213, Section 4.7.3.5. Lines 3-5 – This statement is completely incorrect; there is no evidence for it as argued above. Delete this, or modify as follows *"Chromium absorbed following ingestion exposure to chromium(VI) may be in forms that can reach the systemic circulation and distal tissues, thereby potentially affecting tissues beyond those at or near the site of entry. However, the form(s) of chromium following such uptake is not known."*

	Chapter 5 This reviewer strongly objects to use of a linear low-dose approach for Cr(VI) risk assessment given the clear evidence for a threshold mechanism due to extracellular reduction of chromium at doses of relevance to human exposure via drinking water. The conclusion by EPA of a mutagenic action of chromium – most of which is based on cell culture data where chromium exposures and other parameters were extreme and where metabolism and intracellular exposure are far different than in vivo exposures – should not be the sole basis for use of this standard model which ignores the compelling toxicokinetic data summarized in Chapter 3 of this draft. More importantly, as discussed above for Chapter 4, Cr(VI) is unlikely to act via a mutagenic MOA in vivo, and requires extraordinary experimental manipulation to be positive in cell culture and in vivo mutagenicity studies. While it is clear that Cr(VI) can cause certain forms of DNA and chromosomal damage or other changes, it is not clear whether any of these is pre-mutagenic, and the in vivo data argue strongly against a mutagenic MOA under physiological conditions and normal routes of exposure. The document must more clearly differentiate between genotoxicity – i.e., damage to DNA or chromatin – and mutagenicity – or frank mutations that may result from DNA damage. Chromium(VI) can induce DNA damage but is a very weak mutagen at best, particularly in vivo. It is far more likely, and most consistent with all available data, that chromium(VI) acts via a non-mutagenic mechanism that involves a clear threshold – two threshold actually, one of which is extracellular and chemical involving reduction of chromium(VI) to chromium(III) and the other of which is biochemical and involves a threshold for cellular effects that lead to cell damage and cell death, resulting in turn in tissue proliferative responses that ultimately increase tumor risk via well known mechanisms of repeated tissue injury, compensatory cell proliferation and re-population. Given this most likely MOA based on a synthesis of several decades of chromium research, it is therefore inappropriate to use a linear low-dose extrapolation model for assessing risk via the ingestion route of exposure. It is clear from the animal and human studies that there is a threshold for in vivo effects that is based on the strong reducing capacity of the GI tract following oral exposures, and that at normal drinking water concentrations this will effectively protect from any in vivo exposure to Cr(VI) as Cr(VI). Thus, a more appropriate risk assessment method would be to do dose-response modeling from the 2008 NTP study and the more recent ACC-sponsored 90-day MOA studies, and then use an approach similar to that for the RfD to calculate, with appropriate safety factors, a drinking water MCL that is protective based on threshold mechanisms. This should be done for Cr(VI) rather than the current MCL that is for total chromium, but it is likely that an MCL in the range of 50-100 ppb is going to be fully protective, including several uncertainty factors that separate it from the departure point of any likely human health effects for even the most sensitive individuals. Chromium is an excellent example of an opportunity to apply the concept of evidence-based risk assessment -- which the EPA has claimed to be promoting for many years but has not, to date, actually applied in any meaningful risk assessment -- since there is a strong and compelling argument for use of a non-linear, threshold-based mechanism for chromium that logically leads to a real-world risk assessment that is based on that mechanism. Setting aside the 1987 Zhang and Li study and subsequent re-analyses as per

	the arguments above, there is not a single credible epidemiology study linking exposure to chromium via ingestion with cancer risk or any other long-term health effects, including in the extensive occupational epidemiology literature which includes several decades of extremely high-dose exposure cohorts. And even taking into account the Zhang and Li study, there is not a single peer-reviewed report linking any health effects to chromium(VI) at levels within a hundred-fold of likely environmental exposures via drinking water. The NTP 2008 animal data showed evidence of a cancer increase only at the highest doses, and the more recent ACC studies demonstrated hyperplasia consistent with a non-mutagenic MOA, which is also consistent with a threshold mechanism and which argues against developing a linear cancer slope factor from those data. **Chapter 6** There is considerable disconnect between the conclusions provided in Chapter 6 and the more considered and detailed discussions of the primary data in the previous chapters. The language should be modified to reflect this understanding such that the conclusions and their application to risk assessment of chromium(VI) follow logically from the scientific evidence. Specific edits: • Page 235, Lines 18-19 – "... *resulting in substantial, and in some cases complete reduction of hexavalent chromium to trivalent chromium depending on the concentration, dose, and precise route and method of exposure.*" • Page 235, Lines 21-26 – "*The extent of absorption of chromium from ingesting hexavalent chromium appears to be determined by both the solubility..... in the GI tract, but ingestion of both trivalent and hexavalent chromium results in systemic uptake of chromium. Trivalent chromium does not readily cross cell membranes except as part of certain organic complexes. Hexavalent chromium, if absorbed systemically, can easily ....*" • Page 235, Lines 27-29 – "*Chromium absorbed systemically from the gut following ingestion of hexavalent chromium is distributed throughout the body. .... If hexavalent chromium is absorbed without extracellular reduction, it can cross cell membranes and, once inside the cell, ...*" • Page 236, Line 3 – "*Chromium absorbed systemically following hexavalent chromium ingestion is eliminated primarily in the urine as trivalent chromium.*" • Page 239, Lines 11-12 – delete the phrase "*.. and evidence of an association between oral exposure to hexavalent chromium and stomach cancer in humans.*" This is a significant statement to make, and as discussed above in reference to Chapter 4, the Zhang and Li studies (1987, 1997) should not be used to assess human cancer risk for oral exposure to chromium. • Page 240, Lines 12-14 – As noted for Chapter 5 above, there is considerable concern with the default choice of a linear low-dose extrapolation model for cancer risk.

Nordberg	EPA has presented and synthesized present knowledge about non-cancer and cancer hazards for hexavalent chromium. However, some further literature could be included in the document and paid attention to i.e, Langård and Costa Chapter 24 Chromium In: Nordberg GF, Fowler BA, Nordberg M and Friberg LT (Eds.) (2007) Handbook on the Toxicology of Metals, 3rd edition, Elsevier 487-510. Other chapters of interest e.g.,Chapter 10 Carcinogenicity by Ke, Costa and Kazantzis page 177-196. One chapter (14 by G. Nordberg and B A Fowler) deals with Risk assessment pages 281-301. A comparison between criteria for classification of carcinogenicity should be done between IARC, EU and USA. Hexavalent chromium is classified as a human carcinogen. This evaluation is also taken by USEPA for inhaled hexavalent chromium and related lung cancer. It should be highlighted that for some metals e.g., arsenic it has been reported in the scientific literature that oral intake i.e., via drinking water also can give rise to lung cancer though oral intake mostly is referred to cancer in the oral cavity or gastrointestinal system. My question is if the lung was studied in the NTP studies or any of the animal studies reported in the given report? Same question goes for the epidemiological studies that are cited? Table 3-7 page 30 reports levels of chromium in female controls both in kidney and in bone. It is not easy to find any comments on this in the document. Is there any analytical problem in this study?
Patierno	I will offer a response to this question in the form of general comments regarding specific sections of the Review in order of appearance in the text. In taking this approach my comments will also directly address questions (A)1-4 and (B)1-5. Page 7: The Environmental Protection Agency (EPA) should not be referencing a 2006 review article by Costa and Klein to site background on environmental chemistry. This review article was not a critical review of the environmental chemistry of chromium. Even if the general background in that review article is accurate, The Toxicological Review of Hexavalent Chromium (TRHC) should cite primary references from chemical or environmental journals or compendiums. Also, this paper is mis-labeled in the reference list as a 2008 paper. [Correct reference is "Toxicity and Carcinogenicity of Chromium Compounds in Humans" Crit. Rev. Tox.: 36(2):155-163, 2006]. Moreover, the premise of this review article [in essence that even very low exposures to any form of CrVI, including in drinking water, can cause virtually any type of cancer in virtually every organ, as well as plethora of assorted other diseases], and the preceding review article on which it was largely based ["Toxicity and Carcinogenicity of Cr(VI) in Animal Models and Humans" Crit. Rev. Tox.: 27:431-442, 1997], should not be universally accepted by the EPA without critical evaluation. Much of the epidemiological methodology applied in these papers is flawed. In these papers, the author(s) repeatedly and selectively tabulated whatever instances could be found in any of the many epidemiologic studies of chromium, of an elevated Standard Mortality Ratio (SMR). These were presented with no mention of the fact that most of these instances

	were small, non-statistically significant elevations (likely to be random fluctuations due to the large breadth of the studies), which were either ignored or discounted by the original authors because of confounding factors. The paper also failed to take into account that many of the small, non-statistically significant elevations in some cancers in one selected study, were counter-balanced by either no elevation or decreased SMRs in other studies. This "tabulation" approach does not constitute a true meta-analysis and is also statistically incorrect. There are also additional reasons that the EPA should be circumspect about citing either of these articles. The 2006 article, and its preceding counterpart published in 1997, were written and published at a time when the senior author was actively engaged as an expert witness for the plaintiffs in high-profile hexavalent chromium lawsuits. This involvement was not disclosed in the 1997 article, which was focused on attempting to implicate low dose exposure to CrVI in a broad array of human cancers. In the Acknowledgements section of the 2006 article there is partial disclosure that production of the paper was paid for in part by Baron and Budd. In fact, Baron and Budd is one the law firms with whom the senior author was under contract with as an expert witness for the plaintiffs in an active lawsuit. This article specifically tried to implicate CrVI as a human drinking water carcinogen even at very low doses, as well as suggesting that exposure to CrVI causes a broad array of other diseases, including neuropsychiatric problems, for which there is no support. If the EPA is going to site these review articles it is critical that EPA conduct an independent critical review of every paper sited in these review articles. In the latter scenario it is certain that EPA will reach a different conclusion. I am not of the opinion that a scientist who serves as an expert witness should have to disclose all litigation-related work in all scientific publications, particularly not in reports of original laboratory research into mechanisms of action, or even in review articles that give an unbiased evaluation of the existing literature, especially as it relates to basic mechanisms of action. Indeed, in the world of chromium toxicology it is hard to find experts who have not participated in some sort of chromium-related litigation. However, these two articles do not merely describe original laboratory research or present an unbiased review of the literature (note that part of the 2006 review article is a recapitulation of an already published journal article on UV light and chromium exposure). These two particular articles are essentially position/opinion papers, with speculative declarations that even very low dose exposures to soluble CrVI can cause virtually any kind of cancer (and other disease) in virtually any organ, a theory of obvious benefit to any plaintiff's case in chromium-related litigation, but one that is not supported by either epidemiological studies or in vivo animal studies. Page 20-21: Although the draft TRHC frequently describes each specific study in this section and offers a conclusion/interpretation, the TRHC discussion of the Donaldson and Barrera paper ends with a reference to Table 3-1 with no summary. There is important information in Table 3-1 that strongly supports the capacity of gastric juice to rapidly reduce CrVI to CrIII. Note that the uptake of Cr in intestinal rings was virtually identical, whether the starting material was CrIII without gastric juice or CrVI plus gastric juice at pH 1.4. The text at the bottom of page 21 and the top of page 24 seems to be nuanced to cast doubt on the body of work of DeFlora, by using the word "suggested" (second line from

	the bottom of pg 21), and then suggesting that the values of reducing capacity given by DeFlora "should be considered with some caution". This "caution" is based on speculation found in the paper cited (Zhitkovich, 2005), and reiterated in the 2006 Costa and Klein article (from where the draft TRHC apparently drew its language). This speculation is addressed in the supplementary materials under "Public Comments". The TRHC and the EPA should not cast doubt on the body of work by DeFlora, based on unsubstantiated speculation. Page 24-5: The TRHC should recognize and illustrate the main point of the absorption studies cited: no matter whether the original starting material is CrVI or CrIII there is limited absorption and little retention of either: fecal recovery in rats was 98% for CrIII and 97.7% for CrVI (pg 24) and in humans was 99.6% for CrIII and 89.4% for CrVI. Pretreatment of CrVI with gastric juice completely inhibited absorption of CrVI after direct perfusion into the small intestine. On pg 27 another study indicates that 99% of CrVI is recovered in feces using rats gavaged with CrVI. On pg 28 another study indicates that maximal uptake after gavage of rats with CrVI occurred in liver and was only 1%. Absorption in other organs was in the range of 0.1 to 0.2%. It is important to note that in all of these absorption studies, including drinking water studies, the increased tissue distribution was only observed after chronic administration of more than 5 ppm. Many of the studies used greater than 100ppm. The main point that there is very little absorption and retention of Cr even after administration of CrVI. Page 26: It is incorrect to state that absorbed "hexavalent" chromium is distributed throughout the body. Few studies actually speciated the Cr found in organs distal to the route of administration and even extremely large doses of CrVI, large enough to saturate reduction in the stomach and GI tract, do not deliver much more than trace amounts of CrVI to most distal organs because of the vast reducing capacity of blood components. The vast majority of Cr reaching distal organs arrives as CrIII. The TRHC should make this absolutely clear. Page 34: The TRHC should provide an accurate summation of Table 3-8 which compares tissue chromium after ingestion of a very large, gastric reduction-saturating dose (12.9 mg/L) of either CrIII or CrVI. The only "organ" that showed consequential increased levels of Cr after CrVI compared to CrIII was blood. Most of the other organs exhibited only trace amounts of Cr, even after this huge dose of CrVI, except for the intestine which showed significant and nearly identical increased Cr concentrations after both CrIII and CrVI. This supports the conclusions of the supplementary data in the Public Comments showing that some sections of the intestine (jejunum for example) are sites of Cr accumulation, regardless of whether the source material is CrIII or CrVI. Page 35: Note that there is no increased accumulation after 8 weeks of exposure compared to 4 weeks of exposure, even at the enormous dose of 130 ppm (mg/L). Page 36: The NTRC should not interject the commentary statement: "indicating that a portion of the CrVI escaped intracellular reduction in the GI tract and became bioavailable for systemic distribution". Like almost every other study this study measured total tissue Cr and did not speciate tissue Cr, and the NTRC therefore cannot speculate on what the form of Cr was that reached the tissues. Note in Table 3-8 the accumulation of Cr in the intestine and blood after CrIII.

	Page 37: Note the obvious threshold of increased bone and kidney concentrations after 10 ppm compared to all lower doses, as well as estimated body Cr burdens. Note that in liver there is a significant Cr burden even after ZERO CrVI exposure. Note the strangely compressed scale of the Y axis: even the increase in females at 10 ppm is only an increase from 0.3 to less than 0.5. These data demonstrate the exact opposite of the conjecture on page 36: there is a clear threshold of accumulation indicating saturation of reductive capacity. Pages 38-39: Virtually every study shows the same thing. The NTP study used a "low dose" that is already higher than the 10 ppm in the Sutherland study. What is being referred to here as a "dose-dependent" increase is already supra-saturation of gastric reductive capacity. What these studies really show is how little Cr is absorbed, even in tissues that are directly exposed (glandular stomach and forestomach), even after massive doses are administered. Page 41: It is inappropriate to make such an unqualified statement as found at the top of this page: "Hexavalent chromium is capable of crossing the placenta". This is only true in the highly contrived circumstances referenced below the statement wherein pregnant mice were given an IV injection of a massive dose of CrVI (10mg/kg). Page 42: The TRHC does a good job describing the bioavailability and kinetics of Cr absorption in humans after CrIII, CrVI in OJ and CrVI. It correctly acknowledges that the CrVI-OJ was completely reduced and that even the full dose of CrVI was insufficient to overwhelm the reducing capacity of blood. The potential explanations offered are correct but need to add another possibility. Often overlooked is the fact that not all CrIII is alike. Anyone who works with CrIII in the laboratory knows that it undergoes aging in aqueous solution, even visibly changing color with time after solubilization. It is possible that CrIII generated from newly reduced CrVI (as in the CrVI-OJ) may have some different biological parameters than straight CrIII made up in water and allowed sit for a couple of days. In fact overall absorption of newly formed CrIII may be higher than aged CrIII, possibly as a function of its ability to form complexes with biological ligands that may alter its absorption potential. Page 49 bottom: It is inaccurate to state that "CrVI distributes to other tissues, notably the blood, kidney, and liver." Except for the cases of treatment with extreme doses, or use of pathways like intra-intestinal instillation or IP injection, the vast majority of Cr that arrives at distal tissues is CrIII. Once again, it is critical that TRHC make that fact clear, otherwise it gives the appearance of non-objectivity. Page 50, last paragraph: The TRHC should not simply reiterate speculation that is found in the papers it cited (Zhitkovich 2005, Costa and Klein 2006), in suggesting that the mutagenicity of Cr may be underestimated in cultured cells because of lower levels of intracellular ascorbate when cells are cultured in absence of added ascorbate. Indeed, it is just as likely that the mutagenicity of CrVI in cultured cells is grossly overestimated, because the lack of ascorbate in the extracellular medium allows CrVI to persist in the extracellular medium thereby maximizing its uptake as the hexavalent oxyanion. At the very least the TRHC should discuss both possibilities and not give the appearance of bias. Page 58: It is inaccurate to state that model simulations "imply" that some CrVI escaped reduction in the stomach. This is circular reasoning. The "input" data that went into

	formulating these models was based on experiments wherein massive doses of CrVI were administered, doses that would clearly exceed reductive capacity. It is not appropriate to then state that the model simulation "implies" that some CrVI escaped reduction, as though the model now supports a novel biological observation. It would be completely expected for the model to predict that scenario since it would logically emanate from the very data that was used to formulate the model. It is critical that the TRHC indicate that these models do not apply to, or accurately predict, the toxicokinetics of low, environmentally relevant doses of CrVI. The discussion in the THRC does become more balanced on page 64 where the non-linear aspects of CrVI uptake, reduction and bio-distribution are given some weight. Page 66: This section (3.6) needs to be completely rewritten as it lends undue weight to an opinion expressed in only one or two papers, at least one of which was written under financial inducement by a law firm with a vested interest in characterizing all Cr, including CrIII, as a potential hazard (see preceding comments). The TRHC needs to not indiscriminately cite speculation found in review articles without more rigorous analysis. Except for those few biased citations it is almost universally accepted that CrIII is an essential element. Page 68: Section 4.1.2, last sentence: This is nearly the ultimate example of how critically important it is for the TRHC to do its own critical analysis of the literature. The paper cited, (Bick et al, 1996) should not be cited under any circumstances and in fact it should be retracted from the scientific literature. Two of the authors, Walter Lack and Thomas Girardi, were two of the 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 (Costa, Bick and Teitlebaum) 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, the draft TRHC does not yet, but should reference the recent work of Dr. John Morgan, an epidemiologist for the California Cancer Registry. He has been tracking cancer incidence in the town of Hinckley CA (the "Brockovich" town) from 1996 to present. He recently published data showing that from 1996 to 2008, not only is there no excess of total cancer or any specific cancer in Hinckley, there are actually fewer cancers than expected. Pages 71-80: The draft TRHC conducts a very thorough depiction of the different interpretations of the Liaoning Province situation. What seems to get lost in the details is the larger picture. This is a Province of a country wherein the background rates of both stomach and lung cancer are high even in non-chromium exposed comparison groups, indicating the presence of other contributing risk factors. This is a situation where exposure is characterized in terms of high dose, long term "yellow water", yet despite this potential significant exposure, the question of whether there is an additional modest increase in risk for stomach cancer hangs on whether a particular industrial area is

	included in the comparison group or not. There is much controversy surrounding the reports of cancer risk in this Province, but after discussing the controversy the draft THRC aligns itself with the method of re-analysis of Beamont et al. The THRC should then also cite the commentary by Allan Smith [Epidemiology 19:24, 2008] which accompanied the Beamont article: although Smith is sympathetic to Beamont's attempt to re-analyze the data, he also describes the extensive weaknesses of the approach. This is not the kind of data that a regulatory decision should be based on. Page 81: The NTP toxicology studies on subchronic oral exposure (Section 4.2.1) are technically well done. The principle issue that needs to not be lost in the detail is that the lowest dose was 62.5 ppm, an enormous concentration of little or no environmental relevance. This is a "yellow water" situation to the extreme. 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. The extensive new data provided by ToxStrategies, described in the Public Comments, needs to be incorporated into the TRHC. Page 84: Again, 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 (see below). 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 (pg 86). Page 87-108: The results 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 emphasized here 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) (Tables 4-12, 4-13, 4-14). 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 should be that these are massive doses and they are eliciting minimal effects. This important concept should not be lost in the mass of detailed results. Pages 109-120: 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 and Klein article, especially in criticizing the work of DeFlora. In point of fact, the results of the NTP assay, and the extensive additional data found in the Public Comments generated by a group of investigators around the country funded by ToxStrategies, 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 profieration, (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. Pages 122-149: For these studies on the potential reproductive toxicities caused by CrVI one can only hope that the TRHC and EPA will remember the 16th century adage of Paracelcus "all substances are poisons, the right dose differentiates a poison from a remedy". These studies show reproductive toxicity at huge doses of CrVI, often given using invasive administration procedure (IP or IV injection), with little relevance to environmental exposure levels. Pages 176-178: The in vivo studies showing DNA damage or mutagenicity in cells peripheral to the point of administration of CrVI were only positive when massive doses of CrVI were administered by gavage, direct instillation, or intravenous injection. Although some studies claim to find mutations in the absence of cytotoxicity these are highly contrived systems: for example eye spots in offspring of pregnant female mice given huge doses (62.5 ppm) of CrVI in drinking water. All studies of mutagenesis in cultured mammalian cells, including human cells, demonstrate that mutagenesis is only observed at doses that produce some degree of cytotoxicity and replication arrest. It should also be clarified in the TRHC that DNA damage and mutagenicity should not be

	equated: while mutagenicity may result from DNA damage, the relationship is not simple or linear and is further complicated by DNA repair. Also, it is unclear whether all forms of DNA or chromatin alterations (collectively termed DNA damage) are pre-mutagenic. For example, in silico studies on DNA-protein crosslinks suggest that under certain circumstances CrIII can serve as binary crosslinking agent between small peptides and DNA. However, in in vitro studies in cultured cells and in in vivo studies, it is not clear what is actually being measured by assays for DNA-protein crosslinks. This phenomenon may in fact only indicate that chromatin isolated from certain cells exhibits a higher degree of condensation during isolation, rendering chromatin proteins more difficult to extract. What appear to be DNA-protein crosslinks can be actually be observed in cells treated with agents that do not participate in or catalyze formation of an actual binary crosslink. Pages 202-214 (section 4.7.3.2): Many of the preceding comments directly address and provide major qualifications to the MOA discussed in this section, including the interpretation of reductive capacity found in the Stout et al report of the NTP assay. It is clear that the EPA is 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-relevent 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 and at high doses that evoke major amounts of cell death. However, 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 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 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 dis-similar to the high-dose rodent assays wherein tumors were only observed at doses that produced chronic and fairly severe tissue damage. This harkens to what is sometimes viewed as a landmark study of lung cancer and occupational exposure to high doses of Cr(VI) by Gibb et al. Occupational exposure to in the chromate production industry was categorized into 4 quartiles. The lowest two quartiles are huge levels of exposure by "environmental" standards, orders of magnitude beyond the even the highest known environmental exposures. The lowest quartile of exposure was essentially a No Effect

	Level (no elevated risk) and the slightly elevated risk ratio in the second quartile was not statistically significant. Interestingly, of the total of 120 lung cancer cases found in chromium-exposed workers, 116 were also smokers. The EPA may be under certain historical regulatory 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 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 etiology of how that particular cancer develops. It will have no relation to any chemically-specific mutations caused by Cr(VI) because Cr(VI) is an exceedingly poor mutagen. Even at the low end of very high doses there is NO MOA because there is NO toxicity, no mutagenecity, 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. If ever there was a textbook case to be made for a "threshold carcinogen", it is Cr(VI).
Rossman	In general, this was a clearly written document. However, my area of expertise is in the mutagenic and epigenetic mechanisms of action of carcinogens, and I found many more problems in those areas than in the rest of the document. I will discuss these in section B2. Here I will mention some errors in the rest of the text. p. 38, end of 1st paragraph reads, "Uptake in guinea pigs did not appear to generally differ from that of rodents". The guinea pig (*Cavia porcellus*) is a rodent. p. 46, section 3.3, refers to transport of the hexavalent chromium oxyanion (for clarity, should this read chromate/dichromate?) by sulfate and phosphate transport systems (should be pleural). It is claimed that this allows accumulation in cells at higher concentrations than the extracellaular concentration. Neither the transport systems nor the evidence for higher intracellular accumulation are referenced. Actually, what allows higher accumulations is the fact that Cr(6) is reduced in the cell to Cr(3), which cannot get out, so what accumulates is Cr(3). p. 201, 1st full paragraph: It is claimed that the key precursor events leading to chromium-induced mutagenicity have been identified in animals. This is not so. It is not even true for mammalian cells in culture. Some ideas have been derived from cell culture studies (but with Cr-damaged shuttle vectors). Almost nothing is known about mutagenicity in animals, and nothing at all is known about the genetic changes occurring in animal tumors or in the target tissues. p. 202. Section 4.7.3. For reasons that will become clearer in my response to B2, this section is very flawed. I will mention just one point here: The confusion between "mutagenic" and "genotoxic" must be cleared up throughout this section (as well as throughout Section 4.5.). The thinking on this issue is very sloppy. A mutagenic mode of action is just that: it requires mutagenesis.

Salnikow	In September 2010, the U.S. Environmental Protection Agency prepared the "Toxicological Review of Hexavalent Chromium" to assess health risks associated with hexavalent chromium exposure. This document will appear on the Agency's online database, the Integrated Risk Information System (IRIS). The existing IRIS file for hexavalent chromium, prepared in 1998, does not consider hexavalent chromium to be carcinogenic by the oral route of exposure. The purpose of the new document is to update the IRIS regarding noncancer and cancer health effects associated with oral exposure to hexavalent chromium after considering the latest scientific evidences. The prepared draft of the "Toxicological Review of Hexavalent Chromium" provides a detailed analysis of the data obtained in several studies carried out in 2008, 2009 by the National Toxicology Program along with other studies, formulates a mutagenic mode of carcinogenic action, and suggests that the reduction of orally administered hexavalent chromium in guts, even in low doses, is incomplete. The draft also calculates a cancer slope factor for humans. Recognizing the importance and relevance of this document, this reviewer needs to address some shortcomings of the document. In general this is a dense document cataloguing many diverse and sometimes controversial studies in the area of hexavalent chromium toxicology and carcinogenesis. Of course the limitations in available experimental data obtained from existing animal models, ongoing investigations regarding the mode of action (MOA) of hexavalent chromium, and uncertainties in epidemiological data make it difficult to prepare a comprehensive document that will fully address public health concerns. Chapter 3 should be reorganized and more emphasis should be given to the role of chromium III in toxico- and pharmacokinetics as well as in biological effects produced by hexavalent chromium. Hexavalent chromium is generally considered a much more potent mutagen and carcinogen than trivalent chromium. Lack of carcinogenic effects observed with trivalent chromium compounds can be explained by poor permeability of cell membrane for this ion. <u>However, considering that the end product of intracellular reduction of hexavalent chromium is trivalent chromium, which may accumulate in tissues, it is important to consider what role intracellularly deposited trivalent chromium may play in chromium toxicity and carcinogenesis.</u> The majority of studies indicate that after intracellular reduction of hexavalent chromium to trivalent chromium, it can form various damaging DNA adducts. These adducts can inhibit the enzymatic activity of DNA polymerases, simultaneously increasing the rate of replication and the processivity of the DNA polymerase, and thereby decreasing its fidelity and causing more frequent errors. The frequency of errors increases with a dose-dependent increase in mutation frequency *in vitro* (Snow, 1991; Salnikow and Zhitkovich, 2008). Unfortunately, it is not clear how applicable these studies are to understanding the effects of trivalent chromium on DNA synthesis and cellular metabolism in vivo because the experiments were done in either in test tubes or in artificial model systems with concentrations far exceeding those obtained through environmental exposure (Snow, 1991; Dai *et al.*, 2009). Numerous attempts have been done to study the distribution and retention of chromium (III) in vivo. Onkelinx studied tissue retention of $^{51}CrCl_3$ in groups of female Wistar rats of various ages (35, 60, and 120 days) after a single intravenous injection of trace amounts of $^{51}CrCl_3$ (Onkelinx, 1977). The study showed that total excretory clearance is the sum of three components: urinary clearance (fu), fecal clearance (fd), and a residual clearance (fs), corresponding to an apparently <u>irreversible deposition of chromium into long term body reservoirs</u>.

	Consistent with the model, ^{51}Cr was found to accumulate with time in several organs such as bone, kidney, spleen, and liver after a single intravenous injection of $^{51}CrCl_3$. These data are supported by those obtained by O'Flaherty (O'Flaherty, 1996) and others indicating that the retention of chromium III by bone, liver, kidney, and spleen is prolonged. Also, to make the toxicological review concise, I suggest eliminating Table 4-21"In vitro genotoxicity studies of hexavalent chromium in nonmammalian cells" and Table 4-24 "In vivo genotoxicity studies of hexavalent chromium in *D. melanogaster*" because these are irrelevant to the MOA of hexavalent chromium in mammalian systems. There are numerous errors throughout the text of the Draft. Some of them are shown at the end of these comments as Errata.
Wise	The Toxicological Review is logical, clear and concise. However, overall the document is inconsistent and thus, EPA has not presented and synthesized the scientific evidence for noncancer and cancer hazard in a clear manner. Some sections, primarily the ones focused on animal data are clearly presented and synthesized. These sections present the primary literature and discuss the merits of each study with balance and insight. Other sections, however, particularly those involving *in vitro* cell culture data and underpinning the mode of action are much less appropriately considered, are not well-presented, and do not synthesize the underlying data very well. Determining a mode of action is a key part of the risk assessment. The Toxicological Review would be a stronger document if it fully analyzed and synthesized the primary literature to ascertain the possible modes of action for Cr(VI). The strengths, weaknesses and data gaps for each could have been highlighted and discussed, and then a rationale for the chosen mode of action presented. However, as presented this approach is not apparent. Instead, as presented, the document gives the impression that the mode of action was pre-determined from a select set of review articles and the best case for that mode of action presented. Decisions appear to have been made to agglomerate all of the genotoxicity data into positive or negative proof of mutagenesis rather than more careful consideration of individual lesions. Confounding factors, such as ascorbate levels, are cautioned against, but inadequately and inaccurately presented and unevenly applied, which undermines confidence that the primary data were adequately considered and contributes to a perception that decisions were predetermined. This perception is strongly reinforced by poor management and citation of the underlying literature and a heavy reliance on a few select review articles that unfortunately miscite the primary literature. As a result, many sections of the Toxicological Review lack clarity, accuracy, synthesis and rigor and the rationale for the choice of mode of action seems predetermined and forced. Each of these factors is elaborated on in more detail below. 1. <u>Unnecessary agglomeration of genotoxicity data</u> The Toxicological Review essentially combines all of the lesions related to genotoxicity (stated on page 212 as "*...including DNA adduct formation, DNA damage, gene mutations, chromosomal aberrations, and micronuclei formation*") into one bundle and refers to them as "mutagenicity". This decision is typically based on the presumption that

	the various genotoxic lesions will ultimately manifest as mutations in the primary DNA sequence. Hence, it is generally recognized that a crosslink or a strand break is not inherently a mutation, but that it may eventually manifest as one and thus, it is a mutagenic event. Based on the aggregation of all of these data, the Toxicological Profile declares Cr(VI) to be a mutagen and proposed a mutagenic mode of action. This approach is consistent with older practice and perception of these genotoxicity assays and it may be a useful approach for a chemical with a limited data set. However, the genotoxicity data for Cr(VI) is a rich data set and deserves a more sophisticated consideration. The Cr(VI) literature often distinguishes in its presentation between mutagenic and genotoxic lesions. The Toxicological Review does not carry forward that distinction and does not explain the rationale for ignoring it. However, it is an important distinction because not all of these lesions are likely to be mutagenic after Cr(VI) exposure. Aggregating these lesions oversimplifies the interpretation of the data and masks the fact that much of the primary data suggest that in actuality, Cr(VI) is a very weak mutagen. Discussing each class of lesion on its own merit with a more careful consideration of the primary literature would have better framed the strengths and limitations of the genotoxicity studies and brought this discussion into a clearer light. The fundamental problem underlying this section is a failure to clearly consider the primary literature to see that Cr(VI) is a weak mutagen when defined as an agent that can directly change the primary DNA sequence. Cr(VI)-induced mutations have indeed been observed in bacteria, cultured cells and animal studies. However, in most cases, one has to experimentally force the mutations to occur by using a high dose, a forced experimental system or a non-physiological exposure route. There is no real synthesis of this literature beyond listing outcomes in a table. The Toxicological Review also considers the results as simply positive or negative. That certainly is one approach; however, it misses the opportunity to consider the data more thoroughly. Some consideration should be given to potency and its potential impact and the robustness of the underlying assays. If the experimental data show that Cr(VI) induces a 2-fold increase in mutations, then the Toxicological Review would call that outcome positive. However, if in that same assay an established mutagen induced a 50-fold increase in mutations, then does Cr(VI) still appear to be a mutagen? Or does it suggest a different mode of action, particularly when the frequency of mutations have not been reported to increase in Cr(VI)-induced human tumors? Does the fact that ascorbate is higher in rodents make these mutations a rodent-specific event? A more careful and thoughtful presentation of the underlying data would have better informed the consideration of this mode of action. In addition, before lumping all of these genotoxic endpoints together as all mutagenic outcomes, a careful review and discussion of the primary mutagenesis literature is needed. That review needs to determine, for Cr(VI), which of the various lesions (e.g. DNA adducts, DNA crosslinks, DNA strand breaks, gene mutations, chromosome damage, etc.) actually occur in cells (for example as discussed below the adducts may not actually form in cells) and to what extent they occur. Then, the review needs to determine which, if any, of these lesions actually lead to gene mutations. The discussion below illustrates that there are reasons to doubt that the various lesions are all mutagenic outcomes. After this

	analysis, those lesions that do form in cells and that do produce mutations could more reasonably be combined into a category of "mutagenicity". Perhaps, the data will indicate Cr(VI) is a mutagen, but, perhaps, the data indicate that one only gets mutations in the DNA sequence when systems are forced experimentally to do so at very high concentrations, due to species specific factors or by non-physiological exposure routes. If the latter were true, this possibility would suggest that mutations are not likely to occur in humans, raising direct implications for the mode of action decision. A more thorough treatment of the primary mutation data is needed to clarify these important points. There is concern that the discussion of some lesions is overstated while others are mentioned but not discussed. The section explaining DNA adducts is greatly overstated and also does not fully consider the primary literature. The section presents a case that implies the status and impact of the various potential adducts are known in cells and *in vivo*. The Toxicological Review even provides a structure of a Cr-DNA adduct. The major concern is that when the primary literature is fully considered, it becomes apparent that these adducts are all based on cell-free systems and no one has been able to clearly identify any specific adducts in cells, whole animals or humans beyond observing tangles of DNA, protein, and Cr that are considered to be DNA-DNA or DNA-protein crosslinks. The primary literature has only measured adduct levels in cells by isolating DNA and then measuring the amount of Cr associated with it or by nonspecific P32 postlabelling. These measures cannot ascertain how or if Cr is bound to the DNA, only that it is associated with it in some way. Some studies have synthesized adducts in cell free systems and applied them to cells, but that does not mean those specific adducts form in the cell. Thus, it is unknown if specific DNA adduct events occur in cells, whole animals or humans. Nonspecific adducts have been detected by postlabelling, but specific adducts remain elusive. To lump these studies together as clear evidence of mutagenicity gives them a weight of evidence that seems premature and inaccurate. Overall, this section is very misleading in its portrayal of the status of adducts as more understood than they actually are. Oxidative damage is also included as evidence of mutagenicity. However, discussion is missing to establish whether this oxidative damage is a direct effect of Cr(VI) causing oxidative damage to DNA and thus, potentially a mutagenic event, or if this damage is actually indirect, resulting from overall oxidative stress to cells caused by high doses of Cr(VI) depleting intracellular antioxidants. Cr(VI)-induced strand breaks are cited as another type of mutagenic event. These lesions are discussed as post-replication-induced breaks. However, the discussion fails to question and discuss whether or not these are actually frank DNA breaks. As the Toxicological Review indicates, the studies have focused on gamma-H2A.X focus production as the measure of breaks. Data indicate that chromatin remodeling may also induce the production of gamma-H2A.X so they may not be frank DNA breaks after all. This possible explanation is missing from the section. DNA-protein crosslinks are included as a type of mutagenicity in the tables and description despite the fact that the document states on page 186 that it is unknown if they

	are mutagenic: *"Tests for the mutagenicity of these crosslinks have proved inconclusive (reviewed in Macfie et al., 2010), but the bulkiness of these lesions indicates the potential for genotoxicity..."* The most consistent genotoxic outcome is the production of damage to metaphase chromosomes manifested as aberrations, sister chromatid exchanges, and micronuclei. The fact that Cr(VI) induces these events was presented but not discussed. This lesion may be the key lesion as it is the most consistent and yet the mechanisms that may underlie it are ignored and not discussed. Chromosome damage could be a mutagenic lesion as assumed. Alternatively, it could be the consequence of epigenetic changes in the cell resulting from Cr binding to centrosomes in the mitotic spindle assembly apparatus or from bypass of the spindle assembly checkpoint. Cr(VI) has been shown to affect centrosomes and the spindle assembly checkpoint and perhaps it causes uneven pulling leading to breaks and errors in chromosome number. Cells with broken chromosomes may undergo apoptosis, while those with increased chromosome number may go on and survive as highly aneuploid cells. Cr(VI) has been shown to induce highly aneuploid cells that can clonally expand and survive. This outcome would not be consistent with a mutagenic mode of action. These concerns above are only magnified by the problems with uneven consideration of confounders and in poor management of the underlying literature described below. Together these factors give the impression of excluding or avoiding different syntheses of the data. More care and balance are needed to discuss and consider the genotoxicity data separately and evaluate if they are mutagenic markers. 2. <u>Uneven application of experimental confounders</u> The perception of bias caused by bundling all of the genotoxicity endpoints together is magnified by an apparent uneven consideration of experimental confounders in the document. It appears that the Toxicological Review does not fully consider and present all of the relevant *in vitro* cell culture data that inform possible modes of action. Instead, selected examples of primary literature that reinforce one point of view are presented. This approach undermines the synthesis of the literature and because of a marked unevenness in presentation creates a perception of bias that should not be part of the analysis. For example, on page 184-185, the Toxicological Review gives the reader the impression that ascorbate-trivalent chromium-DNA adducts have been found to be highly mutagenic. However, the section did not describe the experimental detail that indicate the cells used in the study were abnormal and genetically modified so that they could not carry out proficient DNA repair or apoptosis, or that the cells were not actually treated directly with Cr(VI). These omissions stand in stark contrast to the experimental criticisms the Toxicological Review applies to other cell culture studies. For example, on page 47, the document states: *"Caution should be used in interpreting cell culture data, as the cell culture medium could play a role in hexavalent chromium reduction, confounding the extent of intracellular hexavalent chromium reduction."*

	It then cites a couple of examples where Cr(V) was detected in extracellular cell culture medium. The use of the word "caution" leads the reader to conclude that many *in vitro* studies may be flawed due to this reduction. No explanation is offered as to why this outcome is a problem. No discussion is provided that points out whether this same type of reduction might be expected to occur outside of cells in the body and thus actually be normal. The practical reality is that reducing agents are present in the extracellular fluid and thus, some extracellular reduction probably occurs in the extracellular fluid. This factor is probably not appropriate as a cautionary one and may actually reflect physiological conditions. But, no balanced discussion is provided for the reader to decide if this factor is indeed a concern. A similar unevenness occurs during the presentation of the relative importance of ascorbate. The Toxicological Review states on page 50: *"An additional important note on these biotransformations regards the interpretation and reliability of data from in vitro assays. In vivo, the intracellular levels of ascorbate are quite high (about 1 mM). In contrast, the levels of ascorbate in tissue culture media are quite low since generally it is not added to the media so that the only source is supplemented fetal bovine serum (FBS). With 10% FBS, the level of ascorbate in tissue cultured cells is only about 50 μM which is 20 times lower than that which is found in vivo (Zhitkovich, 2005). Therefore, experiments on mutagenesis and other toxic effects of hexavalent chromium in tissue culture may underestimate its mutagenic, genotoxic, and cell-transforming activities (Zhitkovich, 2005; Costa and Klein, 2006)."* No further discussion is presented. There is no presentation of the primary literature in cell culture showing the impact or lack of impact of this difference, just speculation that it might cause some underestimation. There is no presentation of the primary literature to establish what the ascorbate levels are in the relevant tissues of concern. There is just this one review article (Zhitkovich 2005) with some comment from a secondary review article (Costa and Klein 2006) cited. Closer inspection shows that the Costa and Klein review is actually citing the same Zhitkovich review so in the final consideration, this entire section relies only on Zhitkovich 2005 which, as discussed below in section 3.D., miscites the primary and secondary literature and draws a conclusion the primary literature does not closely support. Thus, the Toxicological Review draws attention to the possible presence of extracellular metabolism and lack of intracellular ascorbate as confounding factors, which it may have used to exclude some cell culture studies. But, by contrast, it makes no mention and expresses no concern about studies done in abnormal compromised cells treated only indirectly with Cr(VI). This discrepancy makes the document and its treatment of the underlying literature seem uneven. Moreover, in its discussion of the impact of ascorbate, the Toxicological Review does not discuss the potential impact of ascorbate differences on the bacterial mutation studies or the possible impact of ascorbate differences on the animal genotoxicity data. For example, in Table 4-23 on page 172, the Toxicological Review indicates a positive effect for mutagenicity in mice after intratracheal instillation. Mice, however, have more ascorbate in their lung tissue. If one accepts the speculation in the Toxicological Review that

	ascorbate–trivalent chromium–DNA adducts form and are highly mutagenic, then the elevated mutations in this study might simply be due to the elevated ascorbate levels in this species suggesting a species specific effect. Such a possibility would explain why there are mutations in rodents but not in human tumors. Regardless of which conclusion is correct, the point is that the Toxicological Review does not appear to apply this confounder it stresses in the *in vitro* work evenly to all studies reinforcing a perception of selective bias. Similarly, the Toxicological Review presents the primary research studies by Quievryn et al., 2003; Voitkun et al., 1998 as showing adduct effects, but both studies used the cell culture medium the Toxicological Review expresses concerns about and neither study addressed the ascorbate concern, but these aspects are not mentioned in the document. The absence of discussion of these confounders in these experiments give the impression that the Toxicological Review does not seem to apply its confounding criticisms evenly. This inconsistent presentation of experimental expectations and application of confounding factors creates a perception of uneven evaluation of the primary literature. Considered together, the language and approach suggest a strong bias against *in vitro* studies and the cautionary language should be removed to eliminate that bias. The discussion about ascorbate needs to be more balanced and thorough and the information better synthesized. The ascorbate section could be removed or if the EPA feels the issue needs to be considered, it should be fully vetted with a discussion of how differences in ascorbate might affect the interpretation of the bacterial mutagenesis studies and the rodent data. The discussion would need to also include the strengths and limitations of the primary literature. The relative merits of data from a primary normal human cell line without vitamin C supplementation versus data from a tumor-derived cell line with ascorbate supplementation would need to be presented and discussed. The various underlying phenotypic issues in cell lines would also need to be considered as a mitigating factor. Similarly, the technical limitations of ascorbate supplementation in culture would need to be considered including how long it is retained by the cell and the impact of its diffusion out of the cell and into the extracellular medium. This discussion would need to include a full evaluation of ascorbate levels in tissues of interest in humans and animals and whether those levels are intracellular or extracellular or both. It should include a full discussion of any data that show whether or not there is an actual impact of different ascorbate levels on outcomes inside the cell from cell culture studies. It should also include a discussion about the fact that ascorbate in the cell becomes depleted over time after Cr(VI) exposure and whether the relevant exposure is when ascorbate levels are normal or depleted. 3. <u>Poor management and citation of the literature</u> The above two concerns are further magnified by the presentation and management of the literature in the sections involving *in vitro* cell culture data and underpinning the mode of action. There is a tendency in the document to overstate the findings of the selected primary literature included in the document, raising questions about whether the primary literature was properly evaluated and weighed. There is inconsistent application of the phrases "*in vitro*" and "*in vivo*" resulting in substantial confusion regarding the underlying

	literature and reinforcing a perception of inaccuracy in the document. There are flaws in citations, extensive direct quoting and long stretches of general paraphrasing of a small number of review articles raising questions about the heavy reliance on those articles and points of view. Finally, there is often a failure to check the underlying primary research studies cited in these review articles reinforcing the perception that the document relies on the review and not the underlying primary research data. Considered together, these aspects raise questions about the process of how the conclusions were drawn, create confusion about whether the primary data were fully reviewed or whether the view of the authors of those few review articles was simply adopted, and raise significant questions about the credibility of the overall evaluation. Each concern is explained in more detail below. A. Overstating the findings of the selected primary literature. There are concerns that the Toxicological Review over-generalizes its presentation of the primary literature, particularly with respect to *in vitro* cell culture studies. One example of this problem is seen in its discussion of the literature concerning DNA adducts where the Toxicological Review states on pages 184-185 that: *"Although the ascorbate–trivalent chromium–DNA adducts are recovered less frequently in vitro due to the low concentrations of vitamin C present in commonly used tissue culture media (Zhitkovich, 2005), these adducts have been shown to be the most mutagenic of all the ternary adducts (Quievryn et al., 2003)." ... "They have been detected in vitro in Chinese hamster ovary cells following exposure to hexavalent chromium, and account for up to 50% of all chromium–DNA adducts. The ternary adducts have been found to cause mutagenic and replication-blocking lesions in human fibroblasts in vitro (Quievryn et al., 2003; Voitkun et al., 1998)."* Thus, the reader is led to believe that ascorbate-trivalent chromium-DNA adducts have been found in cells and these adducts have been shown to be highly mutagenic. Careful examination of the two cited references reveals that the statement in the Toxicological Review quote listed above that states: *"They have been detected in vitro in Chinese hamster ovary cells following exposure to hexavalent chromium..."* is incorrect. Detection of adducts in Chinese hamster ovary cells was not actually presented as data in either paper or mentioned in the text of either paper. The claim is unsubstantiated as presented, which makes it potentially misleading. Furthermore, when one considers the experimental detail in Quievryn et al., 2003 and Voitkun et al,, 1998, one learns that the adducts were synthesized in a cell free system. A sequence of DNA was treated with Cr(VI) and ascorbate in a cell free system. Then, the damaged DNA sequence was administered to cells. The cells then converted the damaged DNA sequence to a mutation that was revealed when the sequence was recovered and sequenced. The detail also shows that the cells used were not normal human cells, but rather a SV40 immortalized cell line. SV40 is known to silence p53 activity, among other cellular and

	molecular changes, thus these cells were unable to carry out proficient DNA repair or apoptosis, as these are normally p53-dependent events. Thus, the studies did not show that these adducts were normally present or able to form inside the cell. They could not account for the fate of the adduct structure after the transfection process and are only assuming it remained intact. The studies did not show that mutations would have occurred normally inside the cell as a consequence of Cr(VI) exposure or as a consequence of these lesions. Moreover, they do not show that these events would have happened in a repair proficient or apoptosis-proficient cell. It could be that the only reason mutations were seen is that the cells' ability to repair or eliminate them through apoptosis was artificially turned off beforehand. An alternative interpretation of the studies could be that one can experimentally force a cell to generate a mutation in response to a Cr adduct if repair and apoptosis are silenced. Indeed, a step forward, but not one that establishes that adducts form or are mutagenic in cells. It is unclear why these two studies were chosen to show that Cr induces adducts in cells. It is remarkable that given the emphasis the Toxicological Review places on the importance of physiologically relevant cell cultures, that it would fail to mention or discuss the integrity of the cell line itself, which in these studies were not robust cells. The document seems to be saying that there is a problem with cell culture studies that have some extracellular metabolism of Cr(VI) or that might not have enough ascorbate, but there are no problems with studies in cells with compromised DNA repair and cell death pathways. There are more examples of this type of exaggeration of the implications of the primary literature in the Toxicological Review. These exaggerations obscure the meaning and applicability of the data and should be corrected. These exaggerations also undermine the integrity of the document and raise questions about its accuracy and process. Other studies in the primary literature may reemerge as more relevant if treated more evenly and these studies should be reconsidered and possibly presented. B. <u>Flaws in citations, extensive direct quoting and long stretches of general paraphrasing</u> One concern is that the Toxicological Review actually appears to directly quote sources without indicating the comments are quotes of the original source. For example, in the Toxicological Review on page 46, lines 13-15, the writing states: *"Studies on the reduction of Cr(VI) by extracts of rat liver, lung, or kidney have found that ascorbate accounted for at least 80% of Cr(VI) metabolism in these tissues (Standeven et al., 1991,1992)."* which is the exact same sentence that occurs in the Toxicological Review's Zhitkovich 2005 reference. That reference states on its page 5: *"Studies on the reduction of Cr-(VI) by extracts from rat lung, liver, or kidney have found that ascorbate accounted for at least 80% of Cr(VI) metabolism in these target tissues (45, 46)."*

	Then on the same page, lines 20-22, the Toxicological Review states: *"Depending on the nature of the reducing agent and its concentration, this process can generate various amounts of unstable Cr(V) and Cr(IV) intermediates (Stearns et al., 1994)."* which is the exact same sentence that is in Zhitkovich 2005. That reference states on its pages 5-6: *"Depending on the nature of the reducing agent and its concentration, this process can generate various amounts of unstable Cr(V) and Cr(IV) intermediates (14-16)."* Neither of these sentences are indicated as being exact quotes of the original source and neither one is attributed to the original source. This omission is a concern as it is important to know when the document is choosing to quote from a source directly. These examples are not the only occurrences of this type of error and the entire document needs to be checked to identify other such problems. In other instances, the Toxicological Review only changes a couple of words in a direct quote and fails to indicate it is a direct quote, which is also unacceptable. For example, the Toxicological Review states on page 50: *"Therefore, experiments on mutagenesis and other toxic effects of hexavalent chromium in tissue culture may underestimate its mutagenic, genotoxic, and cell-transforming activities (Zhitkovich, 2005; Costa and Klein, 2006)."* The underlying reference by Costa and Klein 2006 states on its page 157: *"Thus, experiments on mutagenesis and other toxic effects of hexavalent Cr in tissue culture may underestimate its mutagenic, genotoxic, and cell-transforming activity (Zhitkovich, 2005)."* The quote in the Toxicological Review and the Costa and Klein review differ by only substituting a "Therefore" for a "Thus" at the beginning and "activities" for "activity" near the end. Changing two words does not avoid the need to offset this sentence as a direct quote. As written, it is sufficiently in the original authors' words that it is considered a direct quote. Similarly, in the Toxicological Review on page 46, the writing states: *"Ascorbate is also the fastest reducer in the in vitro reactions, and its rate of reduction at 1 mM exceeds that of cysteine and glutathione by approximately 13 and 61 times, respectively (Zhitkovich, 2005; Quivryn et al., 2001)."* which is the exact same sentence that is in the Toxicological Review's Zhitkovich 2005 reference that states on its page 5: *"Ascorbate is also the fastest reducer of Cr(VI) in the in vitro reactions, and its rate of reduction at 1 mM concentration exceeds that of cysteine and glutathione approximately 13 and 61 times, respectively (48)."*

	Again, this language is a direct quote and needs to be offset in quotation to make that clear. This type of error also occurs with some frequency in the document and needs to be addressed. Next, there are numerous instances when the Toxicological Review extensively paraphrases a review article and the meaning of the original passage is altered to another meaning that was not originally intended resulting in some overstatements and inaccuracies. For example, the Toxicological Review states on page 185: *"Reduction of hexavalent chromium in vitro produces a large proportion of binary trivalent chromium–DNA adducts, but these have not been detected in vivo. It has been theorized that the formation of the ternary adducts described above occurs far more frequently due to the high concentration of ligands capable of complexing with trivalent chromium before it can bind to DNA. (Zhitkovich, 2005)."* Given its general use of "*in vitro*" to mean "in cell culture", the Toxicological Review appears to be stating that binary trivalent chromium-DNA adducts occur in cell culture but not in *in vivo* studies. However, the underlying Zhitkovich review reference actually states that the binary adducts have been detected in a test tube and not in cell culture. Specifically, it states (bold added here for emphasis): *"Reductive metabolism of Cr(VI) in vitro usually generates a large number of binary Cr(III)-DNA adducts (22, 37, 53), but the presence of these DNA modifications* ***in cells*** *has not yet been established. The formation of binary Cr-DNA complexes in cells is expected to be strongly inhibited due to the abundance of intracellular ligands capable of rapid coordination to Cr(III) prior to its binding to DNA."* This error occurs quite often and creates confusion about what the underlying literature is indicating. There is a reference to "Salnikov and Zhitkovich, 2009" in a couple of places and no citation for this reference is provided. In the citation list, there is one reference listed as "Salnikow, K; Zhitkovich, A. (2008)" and another just below it as "Salnikov, K; Zhitkovich, A. (2008)" that looks to be exactly the same reference. These details should be straightened out and the entire reference section rechecked. The occurrence of these various errors in citations undermines confidence in the Toxicological Review and raises significant concerns about process. It gives the impression that review articles formed the basis for the evaluation, rather than primary sources and, with the extensive quoting and paraphrasing, that some articles were simply integrated into the document. The use of these review articles in the document needs to be revised and addressed. C. <u>Confusion due to inconsistent application of the phrases "in vitro" and "in vivo".</u> The data in the Toxicological Review essentially fall into four groups. There are cell-free system studies, cell culture studies, whole animal studies and human studies. To describe these data, the phrases "*in vitro*" and "*in vivo*" are used. To most readers "*in vivo*" is

	thought to refer to studies in the body and so include whole animal and in some instances human studies. By contrast, "*in vitro*" is thought to refer to cell culture studies. There are inconsistencies in the use of these terms in the Toxicological Review as some of the underlying references use "*in vivo*" to mean in cell culture and "*in vitro*" to mean in cell free systems. The Toxicological Review has often failed to clarify the underlying studies and carried the underlying language forward into the review. Two examples of this problem are presented in the preceding criticism, where the Toxicological Review elevated the cells in culture to an "*in vivo*" status, but there are many more occurrences in the document. There are two explanations for this outcome. One possibility is that in some places the Toxicological Review uses "*in vitro*" to mean in cell culture and in others to mean in cell free systems and in some places it uses "*in vivo*" to mean in whole animals and in others to mean in cell culture. The second is that the authors of the Toxicological Review did not realize that the underlying literature meant for "*in vivo*" to mean in cell culture and "*in vitro*" to mean in cell free systems. Regardless of which reason applies, as written the use of the terms is confusing and in some cases, such as the one explained above, misleading. The EPA needs to decide on a definition for these terms, present it, review the underlying literature to be sure they reflect what is meant and then apply them consistently in the document. D. <u>Failure to check the underlying primary research studies in review articles</u>. There was a failure to fully consider the underlying primary research articles in the review articles that are extensively cited. This failure creates a perception that the authors did not read beyond that review article, raising questions about process and whether primary data was evaluated at all. Where the document depends on a review article for its source, the underlying primary literature should be checked to confirm the integrity of the statements. One example of this problem is seen in the passage below from page 50 of the Toxicological Review: *"An additional important note on these biotransformations regards the interpretation and reliability of data from in vitro assays. In vivo, the intracellular levels of ascorbate are quite high (about 1 mM). In contrast, the levels of ascorbate in tissue culture media are quite low since generally it is not added to the media so that the only source is supplemented fetal bovine serum (FBS). With 10% FBS, the level of ascorbate in tissue cultured cells is only about 50 μM which is 20 times lower than that which is found in vivo (Zhitkovich, 2005). Therefore, experiments on mutagenesis and other toxic effects of hexavalent chromium in tissue culture may underestimate its mutagenic, genotoxic, and cell-transforming activities (Zhitkovich, 2005; Costa and Klein, 2006)."* Thus, the Toxicological Review wants the reader to question cell culture studies if they do not contain intracellular levels of ascorbate in the mM range. However, the Zhitkovich review article that this section is entirely based upon miscites the primary literature on this matter and it appears the Toxicological Review did not check it. Specifically, as seen in the passage below from the Zhitkovich 2005 review, the claims about physiological levels of ascorbate being in the millimolar range rely on primary research papers that are its references 53 and 54. The Zhitkovich review states:

	"Under standard tissue culture conditions, A549 and many other human and rodent cells either lack detectable ascorbate or contain it only at micromolar levels (physiological levels are in millimolar range) (53, 54) due to low concentrations of this vitamin in fetal bovine serum and its absence in the most commonly used types of growth media (DMEM, RPMI 1640, F10, F12)." These two references are: *"(53) Quievryn, G., Messer, J., and Zhitkovich, A. (2002) Carcinogenic chromium(VI) induces cross-linking of vitamin C to DNA in vitro and in human lung A549 cells. Biochemistry 41, 3156-3167.* *(54) Salnikow, K., Donald, S. P., Bruick, R. K., Zhitkovich, A., Phang, J. M., and Kasprzak, K. S. (2004) Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress. J. Biol. Chem. 279, 40337-40344."* Examination of these two references shows, however, that neither one offers any data or evidence of physiological ascorbate levels being in the mM range. Salnikow et al. measures the amount of ascorbate loss in cultured cells treated with nickel and cobalt. They do measure ascorbate levels in untreated control cells to determine the background level of their experimental system. But their study does not measure any levels of vitamin C in any physiological setting. Nor does the citation make any reference at all to any study that does. Quievryn et al., treats the human carcinoma cell line A549 with ascorbate and dihydroascorbate and then measures the amount of vitamin C inside the cell under these experimental conditions. But the study does not measure any levels of vitamin C in any physiological setting. The discussion section does make a comment that: *"Human cells in vivo contain a millimolar concentration of Asc (43)..."*. However, that reference 43 is *"43. Meister, A. (1994) J. Biol. Chem. 269, 9397-9400"*, which is a review article concerning glutathione and ascorbate in rodents. It contains no mention of ascorbate in human cells. Thus, the EPA expresses a significant concern about *in vitro* cell culture studies based on a single review article that miscites the primary and secondary literature. This oversight implies that in the preparation of this Toxicological Review, the EPA did not access the primary literature and confirm the secondary and tertiary review articles. If one were to look at primary literature for ascorbate levels, one would find that these claims are overstated. Ascorbate can reach mM levels in the body, but they are not universally mM levels. For example, Slade et al., report lung ascorbate levels of 2.91 to 62.35 mg/100 g (Slade, R., Stead, A.G., Graham, J.A., and Hatch, G.E. (1985) Comparison of lung antioxidant levels in humans and laboratory animals. Am. Rev. Respir. Dis. 131(5), 742-6). This measure can be converted to a range of 165 uM to 3.5 mM. However, these are not intracellular levels, but rather the product of tissue homogenization and so a mixture of extracellular and intracellular sources. Thus, there is clearly variability in levels that span the uM and mM range, indicating that this factor may not be so essential.

	E. Some exaggerations about Cr transport in cells. Of less concern, but certainly in need of being addressed is some of the inaccurate language concerning Cr transport into the cell. In several places, cells are described as being impermeable to Cr(III). This characterization is too strong and inaccurate. Cr(III) will enter cells. It is just a slower uptake process than Cr(VI) uptake as Cr(III) moves by simple diffusion and it requires a higher dose to create the concentration gradient to get in the cell. This language should be adjusted. Also, there is language implying Cr(VI) is actively transported into cells. Cr(VI) does enter rapidly by facilitated diffusion, but it is not an active transport process. These comments should be adjusted. F. Some typographical errors. There is also a mention on page 150 that states: 'As discussed in detail in Section 4.4.2 (Intracellular Reduction)..." It is actually section 4.5.2. In sum, these factors all combine to give the appearance that the mode of action was not fully and consistently considered. To make the document and its conclusions much stronger and more accurate and the rationale behind its decisions more transparent, the following steps should be taken: 1) The EPA needs to separate the genotoxicity literature into discrete endpoints and consider them individually. This consideration should be based on the primary literature, which should be presented in a more careful and coherent fashion so that the reader can understand the strengths, weaknesses and data gaps. 2) Based on that analysis the EPA should choose which lesions are the key lesions and explain the rationale for that choice. 3) Once the key lesions are chosen, the EPA should consider the possible mechanisms of action that may cause those lesions and determine if there are data to support those mechanisms of action. 4) Once the key lesions are identified and the likely mechanisms described, the EPA should explain its rationale for the one chosen to be the mode of action. Of course, non-genotoxic modes of action should also receive similar analysis and presentation. This approach would help the EPA determine the most robust mode of action based on the primary literature. In addition, the EPA should decide what factors are truly confounders of concern and then apply them evenly to all of the literature, reduce its use of secondary and tertiary review articles and improve its management and citation of the literature.
Zhitkovich	In general, I found the Draft to be well prepared and balanced in its presentation of various aspects of chromium-6 toxicology and carcinogenesis. It has a logical structure, leading a reader from the basics of redox chemistry of chromium-3 and chromium-6 and their interactions with biosystems to the detailed description of *in vivo* studies on bioavailability, tissue disposition and finally, toxic and carcinogenic effects. Weaknesses and strengths of the key *in vivo* studies along with the reasons for the inclusion or exclusion of specific findings were also clearly presented. Different sections vary somewhat in their degree of emphasis on the importance of one or another mechanistic aspect of Cr(VI) toxicology, which is also reflective of divergent opinions in the field. As typical for any large document covering a complex topic, the Draft contains some information that is not up-to-date and would benefit from additional editorial work.

	Suggested modifications and corrections: 1) Section 2.1 "*Environmental Sources and Occurrence*" appears to draw a large amount of information from decades-old literature. The analytical approaches for the detection of both total Cr and Cr-6 underwent major improvements in 1980s and the older references to the amount of Cr-6 in various environmental media and biological samples should be looked at with a healthy degree of skepticism and scrutinized for potential overestimations. My specific concerns are related to the included values for Cr-6 levels in soil, freshwater and seawater. All three sets of values are too high for the typical samples from the noncontaminated areas/sites. 2) Table 2-1 "*Industrial uses of hexavalent chromium compounds*" is missing uses of sodium/potassium chromate and dichromate. The addition of information on sodium dichromate is particularly important in light of its testing for carcinogenicity by the NTP. 3) p.14, last para: Cr(III) oxidation to Cr(VI) by atmospheric oxygen can also occur in the presence of calcium oxide (Pillay et al. 2003). 4) Table 2-4 "*Detection limits for methods...*" reports outdated values. The EPA's Method 218.6 for Cr(VI) in water has a detection limit which is ~100 times lower than detection limits listed in Table 2-4. A recent modification of this method affords detection of Cr(VI) at the 0.003 ppb level (Application Update 179 from Dionex). The detection sensitivity of flame AAS is also underestimated. Based on the discussion of work by Levine (2007) on p.16 and other available literature, the detection limit for total Cr in water samples by ICP-MS reported in Table 2-4 is probably lower by a couple orders of magnitude. 5) p.25, lines 5-6: in Donaldson and Barreras (1966), urinary excretion for orally administered Cr(VI) and Cr(III) were 2.1 and 0.5%, respectively (not 2.1 versus 1.5%). 6) Table 3-5 and the discussion of these results appear contradictory. 7) Table on p.39 is confusing: it reports daily doses of sodium dichromate dihydrate in the NTP-2008 study but the ratio mice:rats looks incorrect based on the data in the top two rows. It is also unclear why Cr(VI) consumption was compared between male rats and female mice and not between animals of the same sex. 8) Finley et al. (1996) delivered a Cr-6 dose of 0.005 mg/kg/day, not 0.005 mg (p.45). 9) Section 3.3 describes Cr(VI) reduction by microsomal enzymes in detail on three pages. This degree of attention may create an erroneous impression about the importance of the specialized enzymatic processes in Cr-6 metabolism. There is a strong consensus in the field that Cr(VI) reduction in mammalian cells is primarily accomplished nonenzymatically by ascorbate and small thiols such as glutathione and cysteine. As acknowledged in other sections of the draft, ascorbate alone accounts for reduction of 80-95% Cr(VI) depending on the tissue (Standeven and Wetterhahn, 1991,1992). A combined contribution of ascorbate and thiols is responsible for more than 95% Cr(VI) reduction. These estimates from tissue preparations were confirmed by the measurements of individual reduction rates (Quievryn et al. 2003). It is clearly important to present mechanistic aspects of Cr(VI) reduction but the detailed focus should be on ascorbate and non-protein thiols, not enzymatic systems with a minimal contribution to the overall

	Cr(VI) metabolism *in vivo*. The absence of Cr(V) intermediate during Cr(VI) reduction by ascorbate is especially important. 10) Last sentence on p. 50: The description of vitamin C accumulation by cells is not entirely correct. Cellular accumulation of vitamin C via uptake of its oxidized form dehydroascorbic acid is a *physiological* mechanism that functions in all mammalian cells. It is particularly active in human cells, which leads to very efficient recycling and much lower daily requirements for vitamin C in humans compared to rodents (Nualart et al. 2003, Montel-Hagen et al. 2008). These differences between humans and rodents are relevant for the interspecies extrapolation. 11) Figure 3-6 needs to be modified: a) Depiction of the cation channel with the comment "No effect" could be interpreted as indicating some nontoxic delivery route for chromium. Unlike some other toxic metals, cation channels play no role in uptake of Cr ions and the cation route should be deleted from this Figure. b) Although some Cr(III)-ligand complexes can exhibit a limited ability to enter cells, there is no evidence that they can react with DNA and cause mutagenic/genotoxic ternary Cr-DNA adducts, as shown in the Figure. The Figure should be modified by removing this nonexistent route of DNA damage. c) The route for the formation of DSB by mismatch repair needs to be revised. As demonstrated in a recent study by Reynolds MF et al (2009), ternary Cr-DNA adducts are directly bound by mismatch repair proteins followed by DSB formation in G2 phase without stalling replication forks in the preceding S-phase. 12) Summary Table 4-20 should add the *+(M) designation* for mutagenesis of sodium dichromate in laboratory animal, as demonstrated by Cheng et al. 2000 (this study was later cited in Table 4-23). 13) Table 4-23 "In vivo genotoxicity studies... Mutations section" is missing references to two positive mutagenesis studies in vivo by Itoh and Shimada (1997, 1998). 14) Section 4.7.3.1. *Hypothesized Mode of Action*: a) Ref. to Salnikow et al. (1992) in support of ternary complexes is inappropriate and could be replaced by Voitkun et al. (1998). b) Neither Zhitkovich (2005) nor Voitkun et al. (1998) dealt with "intrastrand DNA-DNA crosslinks". A study by Lloyd et al. (1998) is the only original report describing putative intrastrand crosslinks, which were generated in a buffer solution with massive concentrations of hydrogen peroxide. Tested under the same reaction conditions, essential metals copper and cobalt were even more potent inducers of these presumed crosslinks. There is no evidence for the formation of these crosslinks by chromium-6 in cells or in acellular systems containing its main biological reducers. 15) Tables 4-22 and 4-23 failed to include any references to studies reporting the formation of chromium-DNA adducts in cultured mammalian cells and *in vivo*. This is a critical omission as the presence of chromium-DNA adducts demonstrates a direct DNA-

	damaging mechanism for Cr(VI) genotoxicity. The formation of DNA adducts was briefly discussed in other sections of the Draft. 16) Discussion of a negative report on DNA damage by DeFlora et al. (2008) on pp.206-207: This study found no evidence of DNA damage in forestomach, glandular stomach and duodenum of female SKH-1 mice after a 9-month long exposure to 5 and 20 mg/L Cr(VI) in drinking water. Based on the study by DeFlora et al. (2008), a high safety threshold argument was also made in some of the submitted public comments. The Draft argued that a shorter duration of exposure (9 months vs. 2 years in the NTP study) made the DeFlora 2008 study "infeasible" for the comparison. With the exception of mutations and a potential accumulation of unrepaired damage in a population of the long-lived crypt stem cells, there are no other obvious factors suggesting that the formation of DNA damage by Cr(VI) in the entire duodenum during the first half of the 2-year exposure would be significantly different from damage occurring at the end of the 2 years. However, the study by DeFlora et al. (1998) is uninformative for other reasons. The authors assayed tissues for two forms of DNA damage: DNA-protein crosslinks and 8-oxo-dG (the Draft incorrectly described 8-oxo-dG as a DNA adduct; it is actually a base oxidation product). Both types of damage showed no increases above background in tissues of exposed animals; however, these negative results were predictable based on the technical limitations of their analytical methodologies. Since Cr(VI) tumorigenesis occurred in the duodenum, I will limit my discussion to this tissue. 1) DNA-protein crosslinks: A positive control consisting of mouse duodenal cells treated *ex vivo* with 1.6 mM Cr(VI) (83.2 mg/L) generated a 2.5-fold response. Such a low responsiveness was clearly insufficient to detect DNA damage for exposures with 4.2- and 16.6-times lower Cr(VI) levels in the 20 mg/L and 5 mg/L test groups, respectively, even in the unlikely scenario of no reduction and no dilution of Cr(VI) with stomach juices before reaching the duodenum. Although chronic exposures frequently leads to the accumulation of unrepairable damage, a dramatic increase in DNA-protein crosslinks during chronic Cr(VI) exposures would be not very likely. DNA-protein crosslinks are repairable lesions in mammalian cells *in vivo* and culture (Tsapakos et al. 1983, Sugiyama et al. 1986, Zecevic et al. 2010) with the possible exception of human peripheral blood lymphocytes (Quievryn and Zhitkovich 2000). Furthermore, ongoing proliferation and shedding of cells in the duodenal villi would result in continuous dilution of damage and loss of previously exposed cells. 2) 8-oxo-dG measurements: A positive control generated by exposure of mouse duodenal cells to 1.6 mM Cr(VI) (83.2 mg/L) produced a 3.8-fold increase in the levels of 8-oxo-dG. It is doubtful that this assay sensitivity was sufficient to detect significant increases in 8-oxo-dG levels for even undiluted/unreduced 20mg/L and 5mg/L Cr(VI) concentrations that were used in the treatment groups. 8-oxo-dG as a biomarker of DNA damage has one critical limitation – a

	short lifetime due to its rapid removal by base excision repair. Repair of 50% 8-oxo-dG occur within 30 min and is complete within 2 hr (Lan et al. 2004). Not only would this short lifetime prevent any accumulation of 8-oxo-dG during chronic exposures, but it would also make it very difficult to detect this lesion even after recently ingested water with a sufficiently high dose producing positive responses under *ex vivo* conditions.
Zhu	This EPA's Review is well organized overall and for most part well presented. The literature review is extensive and thorough. However, the Review does not contain a set of clearly-stated criteria under which the literature was searched, critiqued, and synthesized. Specifically, was each published study judged with respect to design (including sample size), exposure assessment, choice of dose metrics, choice of endpoints, adequate dose-response data, dose-response modeling, and positive findings? Whereas some of these criteria may have been used in the Review, the lack of a systematic approach may have compromised the consistency and transparency of this review process. In its independent review of the EPA's IRIS Documents on Formaldehyde and Dioxins, for example, the National Academies of Science and National Research Council have strongly advocated the adoption of a systematic review approach to EPA's IRIS risk assessment process (NAS 2006, 2011). The present Review of Hexavalent Chromium once again demonstrates the need for adopting a systematic review approach.

G2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

Reviewer	Comments
Byczkowski	It seems that up to date (around the year 2009), all important studies (and some unimportant too) have been already covered by this Toxicological Review. However, I strongly suggest that the results of study presented at the workshop by ToxStrategies, Inc., and especially the physiologically based pharmacokinetic (PBPK) modeling by Summit Toxicology, LLP., should be included in the revised Toxicological Review of Hexavalent Chromium document.
Hamilton	See G1. above.
Nordberg	To my understanding there are new experimental studies with more for this purpose fitted doses. It could be worthwhile to await the outcome of these studies to find out whether more appropriate values for both NOAEL and LOAEL will be reported and include these in an appendix if feasible. Any further data obtained in the cited NTP studies should be included and presented to the reader. It would be of interest to know if there are any data on lung cancer or other effects from the NTP studies. During the workshop a number of ongoing studies were presented and it was suggested that they be paid attention to. It is always an advantage to get more and more information and research is always going on.

	In my opinion it is however important to set recommendations for exposure to toxic agents in order to protect humans from developing adverse health effects. It is a human right to be protected from unwanted exposure which also will cause unnecessary worry during the time from alert to protection. People expect regulatory agencies to make evaluations and set exposure limits. Studies underway even if published in peer review scientific journals should be carefully evaluated and scrutinized by EPS´s working group to determine if presented data is reliable e.g., based on a number of factors such as, just to mention a few, how large are the studies and what is the power o the study, analytical procedures that include quality control so data is validated and to be trusted. Based on experience it takes time before data will be available even for ongoing studies. I recommend that IRIS, EPA sets a recommendation based on information presented in the draft document. In case important information which can change any evaluation shows up in time, such data can be included in the final document as an appendix or addendum. It is important in Risk Assessment to keep in mind that any recommendation set for exposure levels values needs to be reevaluated over time because by new techniques e.g., rapid development of usage of "omics" has to be considered. In view of said it is important to draw conclusions now and on data available now and not to wait.
Patierno	The TRHC should absolutely consider the extensive new data being provided by ToxStrategies and presented in part in the supplementary section under Public Comments.
Rossman	Some of these will be presented in section B2, as they pertain to mode of action. It is extremely important that the new information supported by American Chemical Council (performed by ToxStudies and others) should be considered before the final document is completed. They address a number of missing data sets. It is already clear that proliferative increases occur in the mouse duodenum at doses of Cr(6) lower than those that cause tumors. Also, there is evidence for cytotoxicity at these lower concentrations that may be driving the proloferative responses. The fact that Cr is an essential element needs to be addressed. What are the implications for a threshold? It is possible that dietary Cr(6) is significant and should be evaluated. All parts of grain contain Cr(6) and 10% of the Cr in bread is Cr(6) (Mishra et al., Food Chem. Toxicol. 33:393-397, 1995; Soares et al., J. Agric. Food Chem. 58:1366-1370). River waters have a median Cr value [which is probably Cr(6)] of 10 ppb (range <1-30), and even rainwater has a range from 0.14-0.9 (ATSDR, Chromium, Draft for Public Comment, online). A recent meta-analysis of cancers of the G.I. tract among those occupationally exposed to Cr(6), concludes that these workers are not at greater risk than the general population (Gatto et al., Cancer Epidemiol. 34:388-399, 2010). Inhalation exposure usually leads to G.I. exposure, suggesting a possible threshold if the ingested dose can be estimated.

Salnikow	Key issues that should have an impact on the conclusions of the draft of the Toxicological Review of Hexavalent Chromium are: 1) the use of appropriate animal models, 2) an understanding of the chromium carcinogenic MOA, including genotoxic (mutagenic) and non-genotoxic (epigenetic) mechanisms and their interrelations, 3) the co-carcinogenic effects of hexavalent chromium, and 4) the role of iron metabolism in chromium carcinogenesis. Unfortunately, studies to address these issues are either not done or are in the early stages of research. Thus, it is too early to draw any conclusive decisions on risk assessment of hexavalent chromium in drinking water. Are used animal models appropriate for risk assessment? Although the NTP studies provide evidence that oral exposure to hexavalent chromium induced tumors in rodents, the main argument against these studies is that the toxic and carcinogenic effects could be achieved/observed only at high chromium concentrations, which significantly exceed human exposure levels. Also, it is noted that biological effects were seen only at chromium concentrations that overwhelmed the cellular defense systems (reducing capabilities). Ascorbate is a major reducing agent of hexavalent chromium in biological fluids and tissues (see review (Zhitkovich, 2005; Salnikow and Zhitkovich, 2008)). Humans cannot synthesize ascorbate in the body because of a mutation in the L-gulono-γ-lactone oxidase gene coding for the final enzyme in ascorbate metabolism and thus ascorbate is supplemented through the diet. Unlike humans, laboratory mice and rats, used for carcinogenicity assays, are capable of synthesizing ascorbate endogenously. Because ascorbate regulates many cell and tissue functions that are critical for cancer development, the changes in the level of ascorbate should be considered in animal models of choice (Salnikow and Kasprzak, 2005). It is impossible to deplete tissue ascorbate levels by metal exposure in wild-type rodents because the enzyme producing ascorbate will be up-regulated when the level of ascorbate drops below a critical point. To avoid this problem for *in vivo* testing of the toxic and carcinogenic effects of heavy metals, which efficiently destroy ascorbate, an appropriate model is the use of mice or rats that like humans cannot synthesize ascorbate (Kasprzak *et al.*, 2011). Two rodent model systems unable to synthesize ascorbate are available: Gulo-/- mice (Maeda *et al.*, 2000), and a similar rat strain (Mizushima *et al.*, 1984). Our preliminary data show that when Gulo-/- mice were supplemented with ascorbate in drinking water their blood and tissue ascorbate levels were undistinguishable from that in wild type mice. However, ascorbate levels were significantly decreased by metal exposure in Gulo-/- mice but not in wild-type mice, in which the enzyme responsible for ascorbate production was activated in response to metal exposure (ascorbate depletion) (Kasprzak *et al.*, 2011). In this model system we found that the reduction in ascorbate levels increased acute toxicity induced by Ni_3S_2 in Gulo-/- mice and that Gulo-/- mice were more susceptible than wild-type mice to nickel-induced carcinogenesis. Additionally, in tumor transplantation assays, Gulo-/- mice had shorter tumor latency than wild-type mice. After the lag period established tumor growth rates were comparable in Gulo-/- and wild-type mice. Although cancer initiation and development is a very complicated process our results indicate that ascorbate is a potentially important part of the molecular mechanisms of metal carcinogenesis and acute toxicity. Ascorbate is involved in diverse biological activities. Ascorbate is essential for the function of numerous 2-oxoglutarate-dependent hydroxylases. This group of hydroxylases

	includes the asparaginyl and prolyl hydroxylases, FIH-1 and PHD1, PHD2, PHD3, which are responsible for HIFα hydroxylation (Epstein *et al.*, 2001; Mahon *et al.*, 2001; Hewitson *et al.*, 2002; Lando *et al.*, 2002); the collagen prolyl-4-hydroxylases (Myllyharju, 2003), which is critical for extracellular matrix formation; and a new class of histone and DNA demethylases that remove methyl group through hydroxylation (Shi, 2007). We already pointed out that the level of ascorbate is critical for metal carcinogenesis mainly by affecting epigenetic pathway (Salnikow and Zhitkovich, 2008). More recently a link between changes in ascorbate concentration and DNA demethylation of human embryonic stem cells (hESCs) has been identified (Chung *et al.*, 2010). Thus, ascorbate levels have the potential to directly impact the differentiation of hESCs and the reprogramming of somatic cells. Given that ascorbate has diverse cellular functions and ascorbate levels are critical to interpreting carcinogenic effects of heavy metals, the animal models described in the Toxicological Review are not the most appropriate. The results obtained in NTP 2007 studies are consistent with the idea that ascorbate is an important factor to consider. In preliminary toxicokinetic studies in which animals were exposed to chromium in drinking water for 21 days chromium concentrations in the blood of the guinea pigs (which are unable to synthesis ascorbate) was greater than chromium concentrations in the blood of the rats or mice suggesting greater absorption (less reduction) of chromium in guinea pigs. http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox072.pdf I suggest that new studies similar to the NTP studies with several dietary concentrations of ascorbate and lower chromium does (i.e., those more relevant to environmental exposures), be done in ascorbate-deficient rats or mice or both animal models. Additionally, because of more efficient depletion of ascorbate in tissues of Gulo-/- animals by chromium these animal models will show whether Cr(III) and Cr(VI) kinetic that were developed using the wild type animals (O'Flaherty, 1996; O'Flaherty *et al.*, 2001) will be applicable to Gulo-/- animals. This will allow for adjustment of kinetic models, if needed, and identification of new or confirming known compartments of chromium retention.
Wise	Although this review is focused on oral exposures, some insight may be gleaned from the inhalation exposure data. Specifically, the data on mutations in lung tumors for Cr(VI)-exposed workers should be considered. These data show a lack of mutations in those tumors suggesting that mutagenicity as considered as a primary change in the DNA sequence is not a key event in the mechanism of action. They are consistent with the fact that one only sees these types of mutations in mammalian experimental models when one forces them by applying very high doses. These studies are: Katabami M, Dosaka-Akita H, Mishina T, Honma K, Kimura K, Uchida Y, et al. Frequent cyclin D1 expression in chromate induced lung cancers. Hum Pathol 2000; 31 : 973-9. Kondo K, Hino N, Sasa M, Kamamura Y, Sakiyama S,Tsuyuguchi M, et al. Mutations of the p53 gene in human lung cancer from chromate-exposed workers. Biochem Biophy Res Commun 1997; 239 : 95-100. Ewis AA, Kondo K, Lee J, Tsuyuguchi M, Hashimoto M, Yokose T, et al. Occupational cancer genetics: Infrequent ras oncogenes point mutation in lung cancer samples from chromate workers. Am J Ind Med 2001; 40 : 92-7.

	Hirose T, Kondo K, Takahashi Y, Ishikura H, Fujino H, Tsuyuguchi M, et al. Frequent microsatellite instability in lung cancer from chromate-exposed workers. Mol Carcinog 2002; 33 : 172-80. Takahashi Y, Kondo K, Hirose T, Nakagawa H, Tsuyuguchi M, Hashimoto M, et al. Microsatellite instability and protein expression of the DNA mismatch repair gene, hMLH1, of lung cancer in chromate-exposed workers. Mol Carinog 2005; 42: 150-8. Kondo K, Takahashi Y, Hirose Y, Nagao T, Tsuyuguchi M, Hashimoto M, et al. The reduced expression and aberrant methylation of p16INK4a in chromate workers with lung cancer. Lung Cancer 2006; 53 : 295-302. Ewis AA, Kondo K, Dang F, Nakahori Y, Shinohara Y, Ishikawa M, et al. Surfactant protein B gene variations and susceptibility to lung cancer in chromate workers. Am J Ind Med 2006; 49 : 267-73. There are also studies of Cr(VI)-induced neoplastic transformation of cells in culture. These need to be considered and included. In particular, reports by Xie et. al., show that cells must acquire a DNA double strand break repair phenotype to undergo transformation indicating escape from repair may be a key event in the mode of action. These studies are: Patierno SR, Banh D, Landolph JR. Transformation of C3H/10T1/2 mouse embryo cells to focus formation and anchorage independence by insoluble lead chromate but not soluble calcium chromate: relationship to mutagenesis and internalization of lead chromate particles. Cancer Res 1988; 47 : 3815-23. Xie H, Holmes AL, Wise SS, Huang S, Peng C, Wise Sr JP. Neoplastic transformation of human bronchial cells by lead chromate particles. Am J Respir Cell Mol Biol 2007; 37: 544- 52. Xie H, Wise SS, Wise Sr. JP. Deficient repair of particulate chromate-induced DNA double strand breaks leads to neoplastic transformation. Mutat Res 2008; 649 : 230-8. The document only considered mismatch repair, but there are important data showing that other DNA repair pathways must be overcome to induce genotoxicity and carcinogenesis. Double strand breaks and their repair, in particular, are important. The following studies should be added to the repair/DNA double strand break discussion: Xie, H., Holmes, A.L., Young, J.L., Qin, Q., Joyce, K, Pelsue, S.C., Peng, C., Wise, S.S., Jeevarajan, A., Wallace, W.T., Hammond, D. and Wise, Sr., J.P. Zinc Chromate Induces Chromosome Instability and DNA Double Strand Breaks in Human Lung Cells. Toxicology and Applied Pharmacology, 234: 293–299, 2009. Xie H, Wise SS, Holmes AL, Xu B, Wakeman T, Pelsue SC, et al. Carcinogenic lead chromate induces DNA double-strand breaks and activates ATM kinase in human lung cells. Mutat Res 2005; 586 : 160-72. Xie H, Wise SS, Wise Sr. JP. Deficient repair of particulate chromate-induced DNA double strand breaks leads to neoplastic transformation. Mutat Res 2008; 649 : 230-8. Stackpole MM, Wise SS, Goodale BC, Duzevik EG, Munroe RC, Thompson WD, et al. Homologous recombination protects against particulate chromate-induced genomic instability in Chinese hamster cells. Mutat Res 2007; 625:145-54.

	Camrye E, Wise SS, Milligan P, Gordon N, Goodale B, Stackpole M, et al. Ku80 deficiency does not affect particulate chromate-induced chromosome damage and cytotoxicity in Chinese hamster ovary cells. Toxicol Sci 2007; 97: 348-54. Bryant HE, Ying S, Helleday T. Homologous recombination is involved in repair of chromium-induced DNA damage in mammalian cells. Mutat Res 2006;599:116-23. Grlickova-Duzevik EG, Wise SS, Munroe RC, Thompson WD, Wise Sr. JP XRCC1 protects cells against particulate chromate-induced chromosome damage and cytotoxicity in Chinese hamster ovary cells. Tox Sci 2006a;92(2):409-15. Grlickova-Duzevik E, Wise SS, Munroe RC, Thompson WD, Wise Sr JP. XRCC1 protects cells from chromate-induced chromosome damage, but does not affect cytotoxicity. Mutat Res 2006; 610(1-2):31-7. Vilcheck SK, Ceryak S, O'Brien TJ, Patierno SR. FANCD2 monoubiquitination and activation by hexavalent chromium [Cr(VI)] exposure: Activation is not required for repair of chromium(VI)-induced DSBs. Mutat Res 2006;610:21-30. Savery LC, Grlickova-Duzevik E, Wise SS, Thompson WD, Hinz JM, Thompson LH, Wise Sr. JP. Role of the Fancg gene in protecting cells from particulate chromate-induced chromosome instability. Mutat Res 2007, 626(1-2):120-127. There needs to be a stronger and clearer discussion about aneuploidy as a potential mechanism. These studies should be added to that discussion (some are in the document already): Holmes, A.L., Wise, S.S., Pelsue, S.C., Aboueissa, A., Lingle, W., Salisbury, S., Gallaher, J. and Wise, Sr., J.P. Chronic exposure to zinc chromate induces centrosome amplification and spindle assembly checkpoint bypass in human lung fibroblasts. Chemical Research in Toxicology, 23(2): 386-395, 2010. Guerci A, Seoane A, Dulout FN. Aneugenic effects of some metal compounds assessed by chromosome counting in MRC-5 human cells. Mutat Res 2000; 469 : 35-40. Seoane AL, Guerci AM, Dulout FN. Malsegregation as a possible mechanism of aneuploidy induction by metal salts in MRC-5 human cells. Environ Mol Mutagen 2002; 40 : 200-6. Holmes AL, Wise SS, Sandwick SJ, Lingle WL, Negron VC, Thompson WD, et al. Chronic exposure to lead chromate causes centrosome abnormalities and aneuploidy in human lung cells. Cancer Res 2006; 66: 4041-8. Wise SS, Holmes AL, Xie H, Thompson WD, Wise Sr JP. Chronic exposure to particulate chromate induces spindle assembly checkpoint bypass in human lung cells. Chem Res Toxicol 2006; 19 : 1492-8. The following two studies should be added to the clastogenicity results, particularly in light of one reviewer's comments that telomerase may be important as the second paper suggests telomerase does not affect Cr genotoxicity:

	Xie, H., Holmes, A.L., Wise, S.S., Gordon, N. and Wise, Sr., J.P. Lead chromate-induced chromosome damage requires extracellular dissolution to liberate chromium ions but does not require particle internalization or intracellular dissolution. Chemical Research in Toxicology, 17(10): 1362-1367, 2004. Wise SS, Elmore LW, Holt SE, Little JE, Antonucci PG, Bryant BH, et al. Telomerase-mediated lifespan extension of human bronchial cells does not affect hexavalent chromium-induced cytotoxicity or genotoxicity. Mol Cell Biochem 2004; 255: 103-11. Finally, there are misleading comments about DNA-DNA crosslinks. The Toxicological Review states they are unlikely to form *in vivo*. When one studies the underlying review cited as evidence by Salnikow and Zhitkovich, it becomes apparent that by *in vivo* they mean cells in culture or whole animals. Thus, the review implies that Cr -DNA-DNA crosslinks would not be predicted to occur in cells or whole animals, however, data from Josh Hamilton and Karen Wetterhahn show Cr DNA-DNA crosslinks do form *in vivo*. The inclusion of Josh Hamilton's study showing DNA-DNA crosslinks *in vivo* would correct the inaccurate conclusion in the Toxicological Review that these lesions do not occur *in vivo*. I cannot locate that paper in the time frame available, but Josh Hamilton is a reviewer and should be able to provide it.
Zhitkovich	The Draft included information from all major studies that have a significant impact on the main conclusions. It does not list or discuss every study published on Cr(VI) but there was also no systematic exclusion. In Section 4.4, I would recommend adding an important report by Gibb et al. (2000), which is the largest epidemiological study of cancer risk due to inhalation exposure to Cr(VI). While the omission or inclusion of this study does not change the overall conclusion about Cr(VI) carcinogenicity to humans via inhalation, Gibb et al. (2000) provided strong evidence of chromate dose-dependence for lung cancer risk and its independence of the common confounder, tobacco smoking.
Zhu	NA

Chemical-Specific Charge Questions

(A) Oral Reference Dose (RfD) for Hexavalent Chromium

A1. A two-year drinking water study of sodium dichromate dihydrate in rats and mice (NTP, 2008) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Reviewer	Comments
Byczkowski	The two-year drinking water study by NTP (2008) seems to be the most comprehensive from all available chronic bioassays, and thus, it is suitable as the basis for derivation of the RfD. However, there is still some concern regarding the selection of the NTP study for developing of RfD (this document states - C.f. P. 85, L# 1: "*...Urinalysis showed dose-related decreased volume and increased specific gravity, consistent with decreased water intake. NTP (2007) suggested that decreased water intake was due to decreased palatability...*" and then, P.89. L# 34: "*...Drinking water consumption was reduced...*") Thus, the reduced drinking water consumption, and consequently at least partial dehydration, may have increased the osmolality of gastrointestinal fluid, which could be a significant confounder in the chronic toxicity study, even though (according to the quotation on P. 120) the Technical Report by NTP (2008) attempted to dismiss such a concern.
Hamilton	No concerns regarding A1. And A2. This reviewer would point out that the hyperplasia that was chosen as this endpoint, while appropriate for this RfD, is also appropriate for considering the carcinogenic MOA as well, as argued above and below and taking into considering the recently reported ACC studies that should be considered in this regard.
Nordberg	EPA suggests an oral RfD of $9x^{10-4}$ mg/kg-day. The epidemiology studies in Liaoning province, China (p 68-76 in draft report) reported increased incidence of cancer after intake of drinking water contaminated with hexavalent chromium. It is stated in the document to be the only reported human data. That study supports the statement of hexavalent chromium in drinking water to be carcinogenic. It should be explained to the reader why sodium dichromate dehydrate was chosen for oral exposure study. Data in the literature indicates that bioavailability and bioaccesibility depends on species of the compound and also exposure media. Are there any data on hexavalent chromium species in the drinking water in the general environment? The NTP studies that are reported have used doses that are much higher than reported present concentration in drinking water in the general environment. Thus values for LOAEL is identical to lowest administered dose. It has not been possible because of applied doses to

	set a NOAEL.
Patierno	The two-year drinking water study of sodium dichromate dyhydrate in rats and mice (NTP, 2008) is the most thorough and technically well-conducted study available. It is likely the best study available for selection. However, the interpretation of and conclusions drawn from that study need to be re-evaluated in light of the issues raised in my preceding comments and the additional data shown in the Pubic Comments and coming available from a multi-institutional study sponsored by ToxStrategies.
Rossman	This does seem like the best and most complete study to use.
Salnikow	Outside of my area of expertise.
Wise	This study is the proper study based on the available data. The study is flawed because only very high doses were considered in the study, thus, there is concern that it may not reflect events at lower doses. The EPA is in the unique position that a study that repeats the one above and extends it to lower doses is almost completed. The EPA should wait for the final results of that study to make the most informed analysis.
Zhitkovich	The NTP-2008 is the best available study of chromium-6 toxicity via oral exposure and its choice for the calculation of the RfD is scientifically sound and was clearly explained in the Draft.
Zhu	The Review offers EPA's rational for selecting the NTP's two-year drinking water study of sodium dichromate dihydrate in rats and mice (NTP, 2008). EPA's justification includes the lack of reliable epidemiological data, solid design of the NTP's experiment, its controlled exposure regimens, the sensitivity of the endpoint, the availability of dose-response data, and consistency with hypothesized genotoxicity MOA. These justifications are acceptable. There are still merits to include other studies, particularly the 3-month sodium dichromate dehydrate drinking water exposure of rats and mice (NTP 2007) in calculating RfDs. Inclusion of additional and all qualified studies is especially beneficial for better quantifying uncertainties and variations arising from different studies due to different study designs, strain/species of animals, and exposure regimens. As in a systematic review, studies meeting selection criteria should all be included for review and for analysis. Selecting a final RfD then becomes a risk management decision.

A2. Diffuse epithelial hyperplasia in the duodenum of female mice was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Reviewer	Comments
Byczkowski	Diffuse hyperplasia, in itself, is considered to be a physiological (normal) response to several stimuli, as the cells of a hyperplastic growth remain sensitive to normal regulatory control mechanisms. Such a physiological proliferation of cells may be secondary to several pathological factors (e.g., the increased osmolality, changed pH, infection by Helicobacter, etc). Still, the proliferation in "diffuse hyperplasia" is a normal process - although, it may be generated in response to abnormal condition (in contrast to neoplasia, where the proliferation in itself becomes abnormal). On the other hand, the hyperplasia is a common early preneoplastic response to potentially carcinogenic stimuli. Considering its rather ubiquitous and nonspecific character, it is remarkable that even no single case of epithelial hyperplasia was found during the two-year study by NTP (2008) in duodena of as many as 100 male and female control mice. Taking this result at a face value, it may be assumed that the diffuse epithelial hyperplasia in duodena could be an early biomarker of oral exposure to Cr^{+6}, at least in the two-year NTP (2008) bioassay study. However, another end point - the pathological changes in the liver were noted in the same study with significantly increased frequency at the lowest dose employed. Even though they may be considered somewhat less "specific" than the epithelial hyperplasia (because they were observed at low frequency also in controls), but perhaps they could be more relevant to the systemic toxicodynamics of Cr^{+6} - appearing further away from the portal of entry then the hyperplasia and apparently being less sensitive to the potentially confounding effects of the reduced drinking water consumption.
Hamilton	See A1. above.
Nordberg	Perhaps some information on possible effects on the lung should be comment on. The document should give information about solubility of different chromium (VI) species should be given and specifically for the chromium species that have been used in the quoted studies. Soluble salts are mentioned on page 54 under 7 but soluble in what media is not mentioned. The reference WHO/IPCS (2006) Environmental Health Criteria 234, Elemental speciation in Human Health Risk Assessment, WHO, Geneva is recommended to be included.
Patierno	The selection of Diffuse epithelial hyperplasia in the duodenum of female mice as the critical effect for the RfD should be re-evaluated in light of the issues raised in my preceding comments. It must be considered in the context of the non-linear, dose-related issues discussed above regarding saturation of reductive capacity and definitive threshold data for toxicity and carcinogenicity.

Rossman	This seems like an appropriate choice, but it's outside my area of expertise.
Salnikow	Outside of my area of expertise.
Wise	This endpoint is a proper endpoint based on the available data, but is not necessarily a toxic outcome. To allow for better understanding, RfD's for other endpoints should be done and presented including some continuous endpoints. The EPA may conclude this endpoint is the critical effect, but this approach makes the analysis more transparent, open and clear.
Zhitkovich	The incidence of diffuse epithelial hyperplasia in the duodenum of female mice was the most sensitive histological response observed in Cr(VI)-exposed groups and therefore, it was appropriately selected as the critical effect for the RfD.
Zhu	EPA considered seven non-cancer endpoints for deriving RfDs (Table 5-1): chronic liver inflammation in female rats, histiocytic cellular infiltration in the liver of female mice, diffuse epithelial hyperplasia in the duodenum of the male and female mice, histiocytic cellular infiltration in the mesenteric lymph nodes of male and female mice, and cytoplasmic cellular alteration of acinar epithelial cells in the pancreas of female mice. All seven are quantal response from the NTP's 2-year chronic exposure study. The selections were largely driven by the dose-response data these effects exhibited. After dose-response modeling and the estimation of benchmark dose (BMD) for each of these select effects, diffuse epithelial hyperplasia in the duodenum of the female mice was chosen as the critical endpoint simply because it yielded the smallest BMD and its corresponding lower confidence limit (BMDL). It must be noted that the dose-response model for this critical effect was done only after deleting the two highest doses. As a result, the dose-response modeling relied on only three dose level (including the control), leaving little room for any flexible dose-response forms other than the "linear" multi-stage model with a polynomial of 1 degree of freedom. Instead of relying on a select "critical" effect, EPA could report a range of RfDs based on a set of qualified and select effects. As a result, EPA will be able to a range of RfDs, projecting the uncertainty and variation of RfDs arising from man y sources and affording risk management the opportunity to make an informed choice of a final RfD (NAS, 2010). This is important as EPA is moving towards enhancing analysis of uncertainty and variation in risk assessment. To this end, EPA could have benefited greatly by including additional endpoints from this principle study as well as other qualified studies. Potential candidates include histiocytic cellular infiltration in the duodenum of female rats and male mice, histiocytic cellular inflammation in pancreatic lymph nodes of male rats, and histiocytic cellular infiltration in the liver of female rats. EPS considered only the quantal responses in this Review for the purpose of computing RfDs. It is unclear why effects of continuous measurement scale were not considered. Many of these effects show unequivocal dose-response (e.g. Tables 4-12 and 4-13) and seem to be relevant to the hypothesized MOA of hexavalent chromium. The availability of EPA's software BMDS for dose-response modeling and benchmark dose computation makes it practical and useful to consider continuous effects for RfD derivation as well.

A3. Benchmark dose (BMD) modeling was applied to the incidence of diffuse epithelial hyperplasia in the duodenum of female mice to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of diffuse epithelial hyperplasia) scientifically supported and clearly described?

Reviewer	Comments
Byczkowski	The benchmark dose modeling was applied in accordance with the *U.S. EPA Benchmark Dose Technical Guidance Document (2000)* in the prescribed manner. However, the modeled BMD, derived from the diffuse epithelial hyperplasia, was supported only by the three dose-points (including zero dose). In contrast, the pathological changes in the liver were consistently fitted, for both female rats and mice with Log-logistic model which included all the five data points (including controls), and thus, they could be considered to be robust dose-response adverse effects for derivation of BMD. So, the pathological changes in the liver may be used as alternative end points for derivation of BMD.
Hamilton	This reviewer is not an expert on modeling and cannot comment on A3 in detail.
Nordberg	Yes.
Patierno	See answer to A2 above.
Rossman	This does seem like the best and most complete study to use (but it's a bit outside my area of expertise).
Salnikow	Outside of my area of expertise.
Wise	The BMD modeling has been appropriately conducted and clearly described. To allow for better understanding, BMR modeling at 5% and 1% should be done and presented. This approach makes the analysis more transparent, open and clear.
Zhitkovich	BMD modeling and the calculations of the POD both appear to be appropriately performed.
Zhu	EPA should be commended for conducting BMD modeling for multiple effects with different model forms. For the modeling of the incidence of diffuse epithelial hyperplasia in the duodenum of female mice, EPA should provide a more detailed discussion on the limitation of the dose-response modeling (See A2). Uncertainties due to model choice, variation in the shape of seemingly equally well-fit models also can be quantified to a degree by considering multiple benchmark response levels (BMR) for each model. EPA used only BMR=10%. It makes perfect sense to also consider BMR=5% or even BMR=1% when such a choice is supported by the data. This is the general recommendation of EPA's own guideline (EPA, 2000). By doing so EPA would be able to quantitatively demonstrate uncertainty and variations due to the choice of different models

	and different BMR levels. Additionally, EPA should briefly but clearly define the BMD concept and methodology in an appendix to improve the readability for readers unfamiliar with the process.

A4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

Reviewer	Comments
Byczkowski	While the uncertainty factors (UFs) were applied in accordance with the U.S. EPA guidelines, the use of the UF = 10 for extrapolating the toxicity value based on effects in the portal of entry of Cr^{+6} in animals to predict GI effects in humans, seems to be problematic. Thus, both the reductive capacity of human gastrointestinal fluids and the antioxidant protection of human tissues exceed those in mice. So, adequately nourished and hydrated humans should be less vulnerable than mice to the adverse GI effects of oral exposure to Cr^{+6}, rather then 10 times more sensitive, as the U_A=10 may suggest.
Hamilton	No concerns regarding A4. Uncertainty factors are policy decisions, not scientific ones, and we can neither prove nor disprove any of the assumptions on which they are based nor can we accurately determine when and how such factors might be applied. Based on previous EPA doctrine, these seem to be consistent with previous applications.
Nordberg	The reason for chosen UFs is clearly and properly described. It is known that the reduction of hexavalent chromium to chromium three is influenced by vitamin C. This can perhaps be used in setting UFs and thus not only choose the standard UFs of 10 between species and 10 for interindividual differences. Humans can not synthesize vitamin C and are thus depending on vitamin C supplementation. The tested animals i.e., the mouse and the rat both produce vitamin C themselves. In this context a laboratory animal that resembles the human by being depended on vitamin C supplementation might be used in future studies. The concentration of vitamin C in tissues and organs are very important in evaluation of carcinogenic metals. It is likely to be involved in the mechanism in causing cancer and plays a role in the MOA.
Patierno	The Uncertainty Factors must be re-evaluated in the context of the non-linear dose-response data, the clear evidence of thresholds for toxicity and carcinogenicity and the fact that these high-dose, supra-saturation experiments cannot be extrapolated linearly to low or vanishingly small doses.
Rossman	This is outside my area of expertise.
Salnikow	Outside of my area of expertise.

Wise	The rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD are appropriate. It was suggested that an UF for children and those with different conditions be used. People with different conditions (e.g. antacid use) are already contemplated in the UF applied for interindividual variation. Currently, such a factor for children is not included in the EPA guidelines. It could be that the same interindividual variation may apply in that case as well.
Zhitkovich	Two UFs were applied: UF=10 for interspecies extrapolation to humans and UF=10 for interindividual variability in the human population. The interspecies UF was used because there is no available information to quantitatively assess the true differences in chromium-6 toxicokinetics and toxicodynamics between humans and laboratory rodents. There are, however, two biological factors that point to a potentially greater sensitivity of humans relative to mice. The first is related to the fact that telomerase was shown to suppress genetic damage by chromium-6 (Glaviano et al. 2006). All mouse cells express telomerase while only stem cells retain telomerase expression in human tissues. The second factor is the difference in ascorbate metabolism. Human cells actively recycle ascorbate (Nualart et al. 2003, Montel-Hagen et al. 2008), resulting in ~100 times lower requirements for this vitamin by humans relative to rodents. A more economical use of vitamin C by humans also results in lower ascorbate concentrations in the extracellular fluid (for example, as reported for bronchoalveolar lavage fluid by Slade et al. 1993), which would more rapidly detoxify chromium-6 via extracellular reduction. However, No specific information about ascorbate concentrations in the extracellular environment of the duodenum and jejunum of mice and humans is currently available. The interindividual variability in sensitivity to chromium-6 was not studied and the application of the safety coefficient (UF) is definitely appropriate in this case. However, the proposed UF=10 likely underestimates the range of the interindividual variability. The Draft has a brief discussion on the common presence of genetic polymorphism in DNA repair genes as one source of interindividual differences. Four major DNA repair pathways (mismatch repair, nucleotide excision repair, base excision repair and homologous recombination) are known to impact the extent of genetic damage and cytotoxicity by Cr(VI), and the use of UF=10 to account for interindividual differences in the overall DNA repair would assume a quite low degree of variability for each repair process (overall 10-fold variation would result from a very narrow 1.8-fold variation in each process: $1.8^4 = 10.5$). Chromium-6 toxicity can be affected on three levels: 1) differences in extracellular detoxification, 2) differences in cellular uptake and 3) differences in cellular/genomic defense mechanisms. A 5-fold variation at each stage would give a potential 125-fold variation in the general population. A study by Donaldson and Barreras (1966) showed that individuals with pernicious anemia had 4-times higher systemic uptake of chromium-6 due to its lower detoxification in the stomach. Widespread use of antacid medications has a clear potential to diminish reduction rates of chromium-6. No systematic studies on potential variations in chromium-6 uptake have been performed yet, but two human lung carcinoma lines, H460 and A549, displayed a 5-fold difference in chromium-6 accumulation (Macfie et al. 2010). A caveat of using information from these two cell lines is that they are malignant and therefore it is not possible to determine whether their

	differences were present in the initial normal cells or whether it is a side effect of different transformation processes. The Draft correctly stated on p.214 that there is no information about susceptibility of children to chromium-6 toxicity. In this case, it would be clearly appropriate to use additional UF=10 to account for a potential early life susceptibility. If EPA considers it unnecessary, then the exclusion of this UF should be justified in Section 5.1.3. My recommendation would be to use UF=100 to account for the combined effects of the interindividual variability in susceptibility and early life exposures.
Zhu	The use of uncertainty factors (UFs) in this review is well described and is consistent with EPA's guidance documents for RfDs. Exposure in earlier stage of life was discussed.

(B) Carcinogenicity of Hexavalent Chromium

B1. Under EPA's 2005*Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgrd.html), hexavalent chromium is *likely to be carcinogenic to humans* by the oral route of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described?

Reviewer	Comments
Byczkowski	Even though the U.S. EPA (2005) guidelines were applied appropriately, there is no direct evidence of dose-dependent carcinogenicity by orally administered Cr^{+6} in humans (C.f. P. 236, L# 33: "*...EPA concluded that the exposure-response analyses presented by Zhang and Li (1997), Beaumont et al. (2008), and Kerger et al. (2009) are not based on the quality of data that is needed to support a conclusion regarding the presence or absence of a dose-response among the observed cancer rates in these villages. The other epidemiologic studies did not find a significant correlation between hexavalent chromium concentrations in drinking water (or proximity to the source of hexavalent chromium soil contamination) and cancer...*") The classification of hexavalent chromium as "*likely to be carcinogenic to humans by the oral route of exposure*" is based on carcinogeniesis observed only at the highest dose levels employed in animal studies. Therefore, it may, or may not be relevant to humans at environmentally relevant exposure concentrations.
Hamilton	Regarding B1. and as outlined in the detailed comments under G1. and G2., this reviewer is concerned that the evidence for carcinogenicity is not strong in animals and is not supported by human epidemiology. As also noted in comments under G1. and G2., there is considerable concern with statements by EPA in sections of this draft, particularly under Major Conclusions in Chapter 6, regarding evidence for human carcinogenesis based on a single human epidemiology study that in turn is a re-analysis of another study that lacks critical information that would be useful in fully assessing relative cancer risk. However, based on the current criteria for selection of this designation, it appears to be

	consistent with EPA doctrine since there is evidence of increased tumors in animals under certain exposure conditions.
Nordberg	Though it is an American document prepared for US I would recommend also to consult and cite documents published by the United Nations Organizations such as International Agency for Research on Cancer (IARC) and World Health Organization (WHO). Recommended literature is IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 49 (1990), Lyon, France and Chromium, Nickel and Welding 1-677 pages and IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 100 which is in preparation and further information on www.iarc.fr Hexavalent chromium is classified as a human carcinogen. It is not clear to the reader why classification can be different upon different route of exposure.
Patierno	The cancer weight of evidence characterization is not scientifically supported. The conclusion that hexavalent chromium is "likely to be carcinogenic to humans by the oral route of exposure" is not scientifically supportable given the issues raised in my comments above. The non-linear dose data in both the NTP studies and the data preliminarily discussed in the Public Comments clearly demonstrate that the toxicities and carcinogenesis observed at these extremely high, obviously supra-saturating doses, cannot and should not be extrapolated to lower doses. See detailed comments above.
Rossman	Given the fact that there is not enough human data to firmly establish carcinogenicity, but there is animal data, "likely to be" is reasonable but "possibly carcinogenic at high dose" would be more accurate.
Salnikow	Hexavalent chromium has been classified by IARC as carcinogenic to humans (group 1) via inhalation route of exposure based on results obtained in human and animal studies. However, when animals were exposed to hexavalent chromium in drinking water the carcinogenic effects were observed only at very high doses, which are irrelevant to human exposure. These results seems to cast doubt on carcinogenicity of hexavalent chromium via oral route of exposure and yet as I already pointed out in G2 the reason for only high chromium doses producing carcinogenic effect may be stemming from the inappropriate animal models which have higher protective ascorbate levels as compared to humans. Although, more human and animal studies are required to make an informed conclusion the ability of hexavalent chromium to produce tumors makes it likely to be carcinogenic by oral exposure. Another important consideration is that hexavalent chromium is a co-carcinogen and consumption of water with other toxic or carcinogenic compounds will result in unraveling chromium carcinogenic effects at much lower doses. Additionally, people with chronic inflammation of digestive tract could be more susceptible to chromium-induced carcinogenesis.
Wise	The general lack of accuracy in the document in its handling of citing, paraphrasing and considering the underlying literature is of some concern in this presentation. There is a lot of cell culture and animal data showing genotoxicity and clastogenicity, however, the

	motivation for these studies were largely inhalational exposure-induced cancer. It seems if they can support one route, they could support the other, but it functionally means the data underpinning the oral route of exposure is the one NTP study. That NTP study is flawed because only very high doses were considered in the study, thus, there is significant concern that it may not reflect events at lower doses. The EPA requirement for a "likely to be carcinogenic to humans" classification defined as "...appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor "Carcinogenic to Humans." It is understandable why an initial assessment of "likely to be carcinogenic" was chosen as the guidance states: " Supporting data for this descriptor may include: ... an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans". The next possible descriptor is "suggestive evidence of carcinogenic potential". It is indicated as "...appropriate when the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion." It is also said to cover "...evidence associated with varying levels of concern for carcinogenicity, ranging from a positive cancer result in the only study on an agent...". Thus, there is conflicting guidance for Cr(VI) on these descriptors. On the one hand, there is a study of multiple species and genders possibly qualifying it for a descriptor of "likely to be carcinogenic to humans". On the other hand there is only one study showing this outcome making it suitable for a descriptor of "suggestive evidence of carcinogenic potential". In studying the data and descriptors, it appears to be premature to conclude that the weight of the evidence is nearly adequate for demonstrating carcinogenic potential to humans. Moreover, in considering the spirit of the guidelines for the two descriptors, it is clear that "likely to be carcinogenic" descriptor is contemplating that the data concerning multiple species, genders, strains etc. will come from multiple studies not just one. It is also clear that the "suggestive evidence of carcinogenic potential" descriptor is intended for a database with flaws. This database is flawed by the lack of multiple studies and the fact that the one study available relied on very high doses. Accordingly, a descriptor of "suggestive evidence of carcinogenic potential" is more appropriate at this time. However, the EPA is in the unique position to soon have another study that repeats the NTP study is almost completed. The EPA should wait for the final results of that study to make the most informed analysis. If it too shows tumors at all doses, then the stronger descriptor would be justified.
Zhitkovich	The classification of hexavalent chromium as "likely to be carcinogenic to humans" via the oral route of exposure is supported by evidence of its tumorigenicity in the oral cavity of female and male rats and in the small intestine of female and male mice. An increased incidence of stomach cancers in the JinZhou area (China), which was contaminated with high concentrations of chromium-6 in drinking water, is supportive of the selected classification. Even if the ecological study from China is excluded, the weight of evidence

	from animal studies is adequate to designate hexavalent chromium as "likely to be carcinogenic to humans" via the oral exposure.
Zhu	The descriptor of "likely to be carcinogenic to humans" for hexavalent chromium is consistent with EPA's Guidelines for Carcinogen Risk Assessment. EPA gave a clear description of the hypothesized MOA.

B2. A mutagenic mode of carcinogenic action by all routes of exposure is proposed as the primary mode of action for hexavalent chromium. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for hexavalent chromium that may support an alternative primary mode of action.

Reviewer	Comments
Byczkowski	The positive laboratory results of mutagenicity tests do not prove genotoxicity and are not necessarily biologically relevant to humans exposed *in vivo* to the environmentally relevant concentrations of Cr^{+6}.The mutagenicity results from humans occupationally exposed to Cr^{+6} (Table 4-25) were inconsistent (this document states - C.f. P. 178, L# 14: "*...In general, associations between hexavalent chromium exposure and mutagenicity in workers are uncertain...*") and actually, the results presented in the document did not prove the direct genotoxic mode of action of Cr^{+6} *in vivo*. An alternative, indirect mode of action seems plausible to this reviewer. As discussed in Section 4.5.2., the intracellular products of one-electron reduction of Cr^{+6} *in vivo*, causing oxidative stress and free-radical damage to cellular macromolecules, seems to be responsible for initiating carcinogenicity at relatively low dose levels. Erosion of GI tract mucosa with inflammation that followed at high Cr^{+6} dosage (discussed in section 4.2.1) apparently caused promotion in the lesions, which eventually progressed to benign and malignant tumors. Importantly, such a mode of action implies a threshold phenomenon, *i.a.* due to antioxidant protection of cells.
Hamilton	There is considerable evidence that Cr(VI) is genotoxic in cell culture and in vitro, and under certain extreme conditions it can also be shown to be mutagenic. However, there is far less support that Cr(VI) is genotoxic or mutagenic in vivo by the oral route of exposure at doses of relevance to humans; conversely, there is considerable evidence that there are protective threshold mechanisms that significantly impact the ability of Cr(VI) to reach target tissues and cause DNA damage under physiological conditions. In addition, while alternative mechanisms are briefly discussed, these are essentially dismissed without extensive treatment. As noted in the document, there is not a large literature on alternative mechanisms, but this is largely because, since the discovery of the genotoxic potential of Cr(VI) some forty years ago, most of the field has only focused on this one aspect, and I suspect it would be very difficult to get peer-reviewed funding to study non-genotoxic mechanisms for this toxicant. It is important to note, however, that there are no reports of increased skin cancer under occupational exposure settings, despite the fact that workers until the past few decades were directly exposed to Cr(VI) on the skin to the

	extent that they formed "chrome holes" that eventually healed. Yet this direct application and clear signs of chromium reduction and toxicity directly on the skin produced no increased skin cancer risk. Likewise, the occupational exposure literature only recently investigated the role of smoking status in chromium-related lung cancer risk. The increased risk of lung cancer associated with Cr(VI) exposure is modest considering the exposure levels and duration of exposures that span decades, and virtually all of the cancer cases in the epidemiology studies were seen in smokers, suggesting an interaction but one that is very modest. This in turn suggests alternative mechanisms such as inflammation, oxidative damage, damage-induced proliferation, and other mechanisms not directly tied to the ability of Cr(VI) to enter cells and damage DNA. These should considered and explored in more detail, since they are the basis for many of the assumptions regarding the risk of cancer from oral exposure to Cr(VI).
Nordberg	Again this might be linked to differences among hexavalent chromium species regarding for example solubility in body fluids. Somewhere in the document it should be pointed that iron can reduce hexavalent chromium to chromium three. This has been done in some products. This is touched by on page 48 line 18. The document describes in detail the possible MOA. IARC 1990 stated "... chromium (VI) compounds on the basis of the combined results of epidemiological studies, carcinogenicity studies in experimental animals, and several types of other relevant data which support the underlying concept that chromium(VI) ions generated at critical sites in the target cells are responsible for the carcinogenic action observed" However, also alternatives like DNA- methylation and other epigenetic mechanisms should be considered, because for many metals DNA-methylation is recognized as a possible mode of action also addressed as mechanism for carcinogenicity. Effects on cell signalling and gene expression may also serve as mechanisms involved in carcinogenesis of metallic compounds. See further discussion in review by Davidsson et al, Chapter 5 in Nordberg et al (eds) Handbook on the Toxicology of Metals, p79-100
Patierno	The determination of a mutagenic mode of action by all routes of exposure should be re-evaluated before proposing it as the primary mode of action. Nearly all indices (NTP studies, inhalation studies, mammalian cell mutagenesis studies etc) indicate that carcinogenicity of CrVI is only observed under exposure conditions that evoke cellular toxicity, inflammatory tissue damage, and tissue regeneration. The DNA damage and presumed mutagenicity (actually epigenetic or stochastic selection of cells that survived toxicity) of CrVI is only observed at doses that also cause cell death and tissue damage. In vivo, these effects are only achieved at very large doses that clearly overwhelm the reductive capacity of the oral cavity, stomach and blood components resulting in a sharp threshold of carcinogenesis only at the two highest NTP doses. I have spent more than 25 years studying the molecular mechanisms of CrVI genotoxicity and mutagenesis and I have a deep appreciation for its capacity to interact with cells and alter DNA and DNA replication and transcription. However, just because CrVI is capable of causing DNA damage and what we thought was "mutagenicity" (see above) in carefully contrived experimental systems, does not mean that it does so under physiologically and

	environmentally relevant conditions. It is much more likely that the chronic tissue damage, with accompanying inflammation and subsequent proliferative regeneration, possibly in the presence of unrepaired DNA damage, all of which are only observed at the highest doses, is the principle mode of action.
Rossman	By definition, the "mode of action" (MOA) of a carcinogen is "a sequence of key events and processes, starting with interaction of the agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation" (USEPA, 2005). For mutagenesis to be a carcinogenic MOA, the agent must at the very least cause heritable mutations in mammalian cells. The mutations should be induced in a concentration range with low toxicity (preferably similar to concentrations seen in human exposures), and the mutations should be induced in the target tissues in animal experiments and in humans. Human and animal tumors should also show genetic alterations consistent with the types of mutations induced by the agents, and these should be early events. The information about Cr(6) is lacking for much of these criteria. In fact, the human tumor data support an epigenetic mechanism more than a mutagenic one. Genotoxic is not the same as mutagenic, and sections 4.5 and 4.73 must be completely rewritten, as they consistently confuse these terms. Standard genotoxicity assays were not designed to inform specific modes of tumor induction. With the exception of mutagenesis, these other assays (non-mutagenic assays) do not measure heritable events, but rather measure evidence of DNA damage or its repair. Non-mutagenic assays include chromosome aberrations, micronuclei, comet assays, DNA lesion measurements, and DNA repair assays. These assays are useful for hazard identification or as biomarkers of exposure. They provide only supportive evidence that mutagenesis might be a MOA. DNA damage *per se* does not inform us about eventual heritable change, which is the true issue. Most (but not all) mutagens cause heritable changes in DNA sequences by causing damage to DNA (pre-mutagenic lesions) that is converted to mutation after cell division. Table 4-21 should be deleted, as results in bacteria are not relevant to tumorigenic MOA. A simple statement that Cr(6) is mutagenic in bacteria should suffice (referencing a review such as Klein CB. "Carcinogenicity and genotoxicity of chromium" In: Toxicology of Metals (Chang LW, ed). Boca Raton, FL:CRC Press, 1996, pp.205–219. Table 4-22 represents positive results in a group of assays that measure both mutagenic and non-mutagenic endpoints (including a paper on epigenesis, Klein et al., 2002, which <u>should be removed</u>, as this is not a genotoxic event; neither is disruption of mitosis, which can have many causes). Table 4.22 has neither information on the concentrations inducing positive results, nor on the toxicity of those treatments. This table is also deficient in the most important results in mammalian cells, i.e. mutagenesis. All of the studies reported are mouse lymphoma cell studies, yet later in the document (page 203), reference is made to mutations at the HGPRT locus in "Chinese Hamster ovary cells (V79 and AT3-2)" V79 is not a Chinese hamster ovary cell, it is a Chinese hamster lung fibroblast. In fact, one such study using V79 cells (Sugiyama et al., Mutat. Res. 260:19-23, 1991) shows a modest positive effect only in a narrow concentration range. A CHO line (AA8) showed a small increase (3.4 fold) at a dose

	giving 75% survival (Brooks et al., 2008). There is a fuller discussion of chromate mutagenicity (with references) in: Klein CB. "Carcinogenicity and genotoxicity of chromium" In: Toxicology of Metals (Chang LW, ed). Boca Raton, FL:CRC Press, 1996, pp.205–219. This article points out that chromium compounds are mutagenic in a narrow dose range, possible because of persistent toxicity after treatment (e.g. residual toxicity was seen a week after treatment of V79-derived G12 cells). Chromosome aberrations and DNA strand breaks can occur as a result of cytotoxicity. Dead cells do not become tumors. Unless assays for cytotoxicity are performed, it is not possible to know whether DNA damage occurs in cells that can replicate to form clones. Traditional cytogenetic assays rely on short-term cell survival to generate the mitotic figures necessary for analyses; the long-term viability of these treated cells cannot be determined. Thus, the relevance of this kind of data for carcinogenic MOA is questionable. To measure cytotoxicty, the gold standard is clonal survival, a method that is common in gene mutation assays, but not in other genotoxicity assays. Short-term survival assays, such as MTT, neutral red, and trypan blue, as well as measurements of mitotic index that are commonly used in cytogenetic assays, fail to detect early or delayed apoptotic events. Trypan blue detects only necrosis. MTT and neutral red assays can be delayed to allow time for apoptosis to develop, at which point the results approach clonal survival (Komissarova et al., Toxicol. Appl. Pharmacol. 202:99–107, 2005). As mentioned above, Cr(6) causes delayed residual toxicity (Klein et al. Environ. Health Perspect. 102 (suppl 3):63-67, 1994), and thus clonal survival or at least apoptosis at later times after exposure, are essential in establishing cytotoxicity levels. Normal human fibroblasts show ~80% loss of clonality after a 25h exposure to 2 μM sodium chromate [Vilcheck et al., Environ. Health Perspect. 110 (Sup5):773-777, 2002]. Micronuclei can result from DNA damage or from malsegregation of chromosomes. It has been recommended that this assay should be performed under conditions of high survival (an increase of >90% in number of viable cells) and that markers for apoptosis and necrosis be included [Kirsch-Volders, et al. (2003) Report from the in vitro micronucleus assay working group. Mutat. Res. 540:153-163]. In the case of Cr(6), at lower concentrations, most of the micronuclei are kinetecore-positive, meaning that they arise from malsegregation and not DNA strand breaks (Seoane and Delout, Mutat. Res. 490:99-106, 2001; Figgitt et al., Mutat. Res. 688:53-61, 2010). Those that are kinetecore-negative (arising from chromosome breaks) occurred only at the highest concentrations. Thus, Cr(6) induces aneuploidy rather than DNA damage at lower concentrations (Holmes et al., 2010; Figgitt et al., Mutat. Res. 688(1-2):53-61, 2010). Aneugenesis is caused by alterations in proteins, not DNA, and has thresholds. It should also be noted that the mouse lymphoma assay (MLA), chromosome aberration assay (CA) and micronucleus assay (MN) give a large number of false positives, even compared with the Ames test. Chemicals that are non-carcinogenic after thorough testing in both male and female rats and mice are often positive in these assays (Kirkland et al., Mutation Research 584 (2005) 1–256). The Comet assay detects single and double strand DNA breaks as well as alkali-labile sites. Nucleotide excision repair (NER) and base excision repair (BER) of adducts can create breaks as intermediates. Single strand breaks are quickly repaired and are not regarded as significant premutagenic lesions. During apoptosis, DNA fragmentation into

	segments of 180 base pairs occurs, whether or not the apoptosis was induced by a genotoxic event (Choucroun et al. 2001, Mutat. Res. 478:89-96; Henderson et al.,.1998, Mutagenesis 13:89-94.) Necrotic cells also display DNA damage (Fairbairn et al., 1996 Scanning 18:407-416.). In order to avoid false positive responses, Henderson et al. (1998) suggests that the concentration of test substance should produce >75% viability. In summary, standard genotoxicity assays from hazard identification exercises cannot be used to establish a mutagenic MOA, because these assays do not measure heritable events and because the doses used in such assays are usually too high. Other MOA's have not been adequately considered. These include, for example, selection for Cr-resistance, resistance to apoptosis, and aneuploidy. The evidence for a mutagenic MOI is weak. Mutations can result from DNA damage, but can also be a secondary effect of the loss of mismatch repair, aneuploidy, and other types of genomic instability (in other words, it is a later effect). With the exception of the mouse lymphoma system, Cr(6) is only weakly mutagenic in mammalian cells, rarely giving more than a 3-fold increase in mutant fraction over background levels (in endogenous genes), and in a very narrow (and toxic) dose-range with a strong threshold (reviewed in Nickens et al., 2010). In some cases the "mutations" have been shown to be epimutations resulting from altered DNA methylation (Klein et al., 2002). Since none of the other studies on mammalian cells looked for epigenetic inactivation, this calls into question whether the "mutants" seen are really mutants. These are important considerations for the MOA of Cr(6), since cells grown in the presence of Cr(6) show selection for cells with inactivated mismatch repair (MMR) genes. These cells are Cr(6)-resistant and could be the result of either mutation or epigenetic inactivation (reviewed in Salnikow and Zhitkovich, 2008). Cells with epigenetically inactivated MLH1 (a MMR gene) were seen in human lung A549 cells exposed to Cr(6) (Sun et al., Toxicol. Appl. Pharmacol. 237:258-266, 2009). MMR-deficient cells are mutators (having a high spontaneous mutation rate) and show microsatellite instability. An important consideration for MOA is the fact that chromium-induced lung cancer cells also show epigenetically-inactivated MMR genes (Takahashi et al., 2005). Also against the idea of a mutagenic MOI is the fact, discussed in Salnikow and Zhitkovich (2008), that Cr-induced lung tumors in humans lack p53 mutations, in contrast to lung tumors associated with other agents such as tobacco smoke, and the fact that the few mutations found do not correspond to the types of mutations caused by Cr in *in vitro* systems. The fact that Cr is essential also implies that oral Cr(VI) could supply the necessary Cr, again implying a threshold at nontoxic doses. Also, experiments from the Costa laboratory (Davidson et al.,Toxicol. Appl. Pharmacol. 196:431-437, 2004; Uddin et al., Toxicol. Appl. Pharmacol. 221:329-338, 2007) showing that chromate in drinking water is a cocarcinogen with solar UV, and the implications of this finding, are not discussed. Other problems (by page): Page 176, top of the page, is a good example of the confusion between mutagenicity and other endpoints. The statement "Hexavalent chromium-induced mutagenicity has been demonstrated following oral exposure" is misleading. There is only one mutagenicity assay showing positive effects on eyespots (presumed deletions) in offspring of female rats given drinking water with 62.5 mg Cr(6)/L. The deletions were not confirmed, so the

	eyespots might be epigenetic events (as Klein found with so-called mutants). All of the other assays are for non-mutagenic endpoints, and tend to be negative for drinking water exposure, but positive for gavage (a more toxic type of exposure). Page 178, 4.5.1.2, it is claimed that mutagenicity has been evaluated in humans experimentally exposed to hexavalent chromium. No such studies appear in Table 4-25. The paragraph mistakenly refers to mutagenicity many times. Page 186: It is not ER (excision repair) that is responsible for removal of bulky lesions, but NER (nucleotide excision repair) that is. Reynolds et al. 2004 is missing in references. Page 187, bottom. It doesn't make sense that mice given 0 mg/kg Cr(6) should have a significant level of apoptosis (compared to what?). Page 188: The Dai et al 2009 paper does not measure mutation frequency in human cells. Page 190: 3rd paragraph: The mutational spectrum of chromate is not clear. See the review by Klein referenced above. Page 202: Key Events, #3: The authors skip from discussing mechanisms of DNA damage by Cr to "overall genomic instability which can lead to mutations if not adequately repaired". Genomic instability can occur as a result of other factors besides DNA damage, and genomic instability is not repaired (DNA damage can be, but the repair often leads to apoptosis). As discussed above, toxic exposure would play a role in the selection of Cr-resistant and/or apoptosis-resistant cells. There is no obvious tie-in here between DNA damage and mutagenesis as a key event, since cells resistant to Cr could have arisen by epigenetic silencing (and this may also be a mechanism in resistance to apoptosis). In a sense, this point is recognized in #4, but in postulating apoptosis as a key event, the authors do not seem to realize the implications, i.e. that a toxic dose is needed for carcinogenicity. They suggest that selection for resistance to apoptosis is due to mutations (either pre-existing or Cr-induced) but there is no evidence for this. Besides altered DNA methylation, other mechanisms for the appearance of Cr(VI)-resistance in exposed cells include epigenetic effects via altered histone modification (Sun et al., Toxicol. Appl. Pharmacol. 237:258-266, 2009) as well as reduction of Cr(VI) transport via down-regulation of sulfate ion transporter activity, and resistance to apoptosis via altered gene expression (upregulation of survival pathways and down-regulation of apoptotic pathways) (discussed in Nickens et al., 2010) p. 204-210: What is described as "mutagenicity" is not in all cases. p. 206, end of paragraph 1: It is claimed that there is evidence that Cr(6) induces mutagenicity in tissues at the site of entry and systemically at doses relevant to human exposure. Where is the evidence for this? p. 206, bottom: Again, it is important to note that DNA damage can lead to apoptosis as well as mutation, so it does not necessarily support a mutagenic MOA. p. 207: De Flora et al., 2008, did not look for mutagenesis, they looked for DNA damage.

	p. 208: Neither O'Brien et al., 2005 nor Eastmond et al., 2008 is in references. p. 209: Low (non-toxic) concentrations would not provide selective pressure for Cr-resistant, mismatch repair deficient cells. Especially in vivo, toxicity would be the driving force to stimulate the outgrowth of such cells. p. 209, 2nd. paragraph: This is a lot of speculation (should be, may be). p. 211, bottom: It is claimed that the study by NTP found no evidence of tissue damage or necrosis. Did they look for apoptosis? It is also claimed that most available studies found Cr-induced genetic damage at doses below those that inducing cytotoxicity. This has not been demonstrated, since clonal survival (the only assay that will detect delayed toxicity) was not performed in these studies. p. 212, end: "...giving rise to mutagenicity (including DNA adduct formation, DNA damage, gene mutations, chromosomal aberrations and micronuclei formation" This is nonsense, as is the next sentence. There is no evidence for mutagenicity at the target tissue at all. p. 213: More confusion between mutagenicity and other endpoints. It is not true that other hypothesized MOA's have not been demonstrated. There is actually more tumor evidence for an epigenetic MOA. Thus, the weight of evidence favors the alternative. This is not a numbers game. Epigenetic studies are relatively new, compared with DNA damage and other "genotoxic" studies, so there are fewer studies. p. 214 top: Has EPA concluded this? Then what is the purpose of reviewing this document? p. 238, bottom: More confounding of mutagenesis and other endpoints. <u>Additional papers showing epigenetic effects of Cr</u> Schnekenburger et al., (2007) Chromium cross-links histone deacetylase1-DNA methyltransferase1 complexes to chromatin, inhibiting histone remodeling marks critical for transcriptional activation. Mol. Cell Biol. 27(20): 7089-101. Sun et al. (2011) Comparison of gene expression profiles in chromate-transformed BEAS-2B cells. PloS ONE 6(3): e17982. Doi:10.1371/journal.pone.0017982. Ali et al. (2011) Aberrant DNA methylation of some tumor suppressor genes in lung cancers from workers with chromate exposure. Mol. Carcinogenesis 50(2):88-99.
Salnikow	**MOA. Genotoxic effect of hexavalent chromium.** The draft of the Toxicological review provides a substantial body of information regarding the mutagenic potential of hexavalent chromium and concludes that hexavalent chromium is carcinogenic by a mutagenic MOA. Indeed this topic has been studied extensively. The results of in vitro and in vivo studies provide substantial evidence for the mutagenic activity of hexavalent chromium, which is mediated through the generation of the highly reactive chromium intermediates penta- and tetravalent chromium, reactive oxygen species, and trivalent chromium formed during the intracellular reduction of

	hexavalent chromium. These chromium and oxygen species can react with DNA, leading to oxidative DNA damage, chromium-DNA adducts, DNA strand breaks, and chromosomal aberrations. Despite these studies, the significance of chromium-induced DNA damage in the mechanisms of chromium carcinogenicity is not clear. If DNA damage/mutations are important and a causative factor in chromium-induced carcinogenesis, then these mutations should be frequent in chromium-induced tumors. However, very few publications exist in this respect. In animal studies, De Flora et al. (De Flora et al., 2008) found no evidence of DNA-protein crosslinks and DNA adducts in the duodenum following drinking water chromium exposures. Other available hexavalent chromium drinking water exposure studies that measured mutagenicity in mice also could not detect evidence of micronucleus induction in the blood or bone marrow (Mirsalis et al., 1996; De Flora et al., 2006; De Flora et al., 2008). Analysis of lung cancers from chromate-exposed workers revealed that p53 mutations are not very frequent, with only six missense mutations identified in 4 (20%) of the 20 chromate lung cancer samples (Kondo et al., 1997). There were fewer mutations in the patients with lung cancers who had been exposed to chromate than in those who had not (20% vs about 50%). This study also revealed that there was no association between p53 mutations and the period spent working in chromate factories. It is conceivable that chromium causes genotoxic effects, damage DNA, but this damage is efficiently repaired and do not play any role in carcinogenic effects of chromium. Thus, in order to confirm or refute a possible role of chromium genotoxic/mutagenic effects in chromium-induced carcinogenesis, comprehensive analyses of mutations in oncogenes and tumor suppressor genes in experimentally induced tumors in animals should be done first, followed by more detailed sequence analyses of chromium-exposed human tumors. A feasible study that could be done in a short period of time (assuming that tumor samples collected in the NTP studies are stored and frozen) is **exon only global sequencing** of DNA from chromium-induced rat tumors versus spontaneous tumors (several spontaneous tumors of different origin were observed in NTP 2008 study). These studies will allow comparison of tumor driving mutations versus passenger mutations. **MOA, Epigenetic effects of hexavalent chromium.** Global changes in the epigenetic landscape are a hallmark of cancer. The initiation and progression of cancer, traditionally seen as a genetic disease, is now realized to involve epigenetic abnormalities along with genetic alterations. Recent advancements in the rapidly evolving field of cancer epigenetics have shown extensive reprogramming of every component of the epigenetic machinery in cancer including DNA methylation, histone modifications, nucleosome positioning and non-coding RNAs, specifically microRNA expression (Sharma *et al.*, 2010). Epigenetic effects have also been observed following hexavalent chromium exposure (Salnikow and Zhitkovich, 2008; Arita and Costa, 2009). Increased DNA methylation was observed in the promoter region of the tumor suppressor gene p16 and the MMR gene hMLH1, indicating that chromium can induce epigenetic effects (Takahashi *et al.*, 2005; Kondo *et al.*, 2006). Gene transcription has also been shown to be affected by exposure to hexavalent chromium in vitro via epigenetic mechanisms. Sun et al. (Sun *et al.*, 2009) found alterations in the levels of histone methylation in human lung A549 cells exposed to hexavalent chromium, indicating the capability of these exposures to lead directly to changes in gene expression.

	Taken together, these studies suggest that epigenetic mechanisms may contribute to the carcinogenicity of hexavalent chromium. However, it is not clear whether epigenetic changes produced by chromium exposure are acting alone or linked to chromium genotoxic effects and that both genetic and epigenetic changes are essential for tumor appearance and evolution. More research is needed in this area including the identification of changes in DNA methylation (analyses of frequency of inherited silencing of tumor suppressors in tumors and in miRNA expression patterns following chromium exposure in animal models and humans before any conclusions can be drawn regarding the role of epigenetics in the carcinogenic effects of hexavalent chromium. **MOA. Co-carcinogenic effects of chromium.** Co-carcinogenic effects of hexavalent chromium were reviewed recently (Salnikow and Zhitkovich, 2008). The majority of occupational and probably all environmental exposures to hexavalent chromium occur as co-exposures with other carcinogens. The most common examples of co-exposures occur among stainless steel welders, and among hexavalent chromium-exposed workers who are also smokers. Two reports from the Costa Lab (Davidson *et al.*, 2004; Uddin *et al.*, 2007) provided strong experimental data demonstrating that hexavalent chromium can act as a potent co-carcinogen for UV-induced skin tumors. In both studies, the presence of hexavalent chromium in drinking water caused dose-dependent increases in the frequency of skin tumors in UV-irradiated hairless mice. Hexavalent chromium alone produced no tumors, indicating that it acted a strong enhancer of UV-initiated tumorigenesis. Supplementation with vitamin E or selenomethionine had no effect on hexavalent chromium-mediated enhancement of skin carcinogenesis suggesting that co-carcinogenic effects were not oxidant-mediated. It is noteworthy that the level of chromium in skin directly exposed to UV had significantly higher levels of chromium than underbelly skin that was not directly exposed to UV in mice exposed to UV and 5 ppm K2CrO4 ($P < 0.05$) (Davidson *et al.*, 2004). This raises an interesting question, does inflammation, whatever the source, facilitate chromium accumulation or delay chromium clearance? This is an important and understudied area. The identified co-carcinogenic effects of hexavalent chromium raise an intriguing possibility that much lower doses of chromium could be hazardous under certain circumstances when exposure to chromium in drinking water is combined with other harmful exposures. Another important area of research is an understanding of the role of Inflammation/colitis in hexavalent chromium carcinogenesis. It is well know that at least 20% of all cancers arise in association with infection and chronic inflammation and even those cancers that do not develop as a consequence of chronic inflammation, exhibit extensive inflammatory infiltrates with high levels of cytokine expression in the tumor microenvironment. Aberrant activation of NF-κB and/or STAT3 is found in over 50% of all cancers and renders premalignant and fully transformed cells resistant to apoptosis and speeds up their rate of proliferation, thereby increasing tumor growth. It is extremely important to test whether hexavalent chromium will be more carcinogenic and at lower doses in animals in which colitis was induced, for example by sodium dextran sulfate. **MOA. Interference with iron metabolism.** In rats neoplastic changes were found at sites of tissue contacts with the highest concentrations of hexavalent chromium, i.e. oral cavity. This may be explained by the

	immediate damaging effects of chromium on DNA and other cellular components. At the same time frequent nonneoplastic changes were observed in duodenum of male and female rats and neoplastic changes were observed in duodenum of male and female mice. These data cannot be explained by the direct effect of hexavalent chromium, which should be mostly reduced by the time it reaches small intestine. Considering that in other organs such as liver and kidney which accumulate significant amount of chromium no tumors were observed, it is important to do more research on the mechanism of tumor development in small intestine. Specifically, duodenum is a place where iron absorption takes place. An analysis of ferroreductase expression and iron metabolism will help to shed light on whether an alteration in iron metabolism in this tissue may be responsible for chromium carcinogenic effects.
Wise	This document is supposed to be limited to an oral drinking water exposure. It is inappropriate to extend any finding to "all routes of exposure" in this document and such evidence has not been considered or presented for dermal or inhalation routes. In its defense of a mutagenic mode of action, the Toxicological Review states on page 213 that: *"In addition to the evidence supporting a mutagenic mode of action in test animals, alternative or additional hypothesized modes of action for hexavalent chromium carcinogenicity have not been demonstrated."* There are three concerns with this statement. First, it seems to imply that other modes of action need to be "demonstrated" not simply supported. The frank reality is that no mode of action for Cr(VI) has been demonstrated, even the mutagenic mode of action is only supported and not demonstrated. There should not be a double standard here where the mutagenic mode of action needs to only be supported, while other modes must be demonstrated. The word "demonstrated" should be changed to "supported" to be consistent with the beginning of the passage. The second concern is that the statement is inaccurate. We supported an alternative mode of action to the induction of mutations in our paper that is cited in the Toxicological Review. Specifically, in Holmes, A.; Wise, SS; Wise, Sr., JP (2008) Carcinogenicity of hexavalent chromium. Indian J Med Res 128:353 – 372, we argue that the mechanism for Cr(VI) does not involve mutations in the primary sequence of the DNA as it is a weak mutagen. Instead, we argue for a genotoxic mechanism leading not to mutations but changes in chromosome number and structure. This point of view is not considered much in the Toxicological Review, but is well-supported in the review and does offer an alternative mode of action that should have been discussed. It is as well-supported as the mutagenic mode of action and so it is inaccurate to state other views have not been demonstrated to the extent a mutagenic mode of action has been. The third concern with the statement is that it seems to imply that the approach taken in determining the mode of action was to consider those possibilities suggested in reviews of the literature. Therefore, because there is no review article synthesizing the literature to suggest a mode of action, there are no other modes of actions to consider. A better approach would have been to consider the primary literature and consider some possible modes of action that emerge from the data, but that have not yet emerged as a review article.

	There are other modes of action that emerge and two possibilities are presented below. There are data to support these modes that should be synthesized, evaluated and considered. The mutagenic mode of action as the primary mode of action is not sufficiently scientifically supported or described in the Toxicological Review. Many concerns in the presentation with respect to proper citation of results, bias against cell culture studies, and an incomplete consideration of the primary literature are discussed above. The only approach taken was to consider all of these lesions in bulk as simply all representative of mutagenic events and not consider the possible confounding factors for each that may indicate they are not mutagenic events. A more careful consideration of the primary literature, considering each endpoint on its own merit could argue against a mutagenic mode of action that involves changes to the primary sequence of the DNA strand resulting in mutations. Cr(VI)-induced human tumors rarely contain such mutations and Cr(VI)-induced mutations are most often generated in experimental systems when one artificially forces them to occur by using extraordinarily high doses or systems with compromised repair and cell death pathways or by non-physiological exposure routes. It is unlikely that Cr(VI) is a mutagen at low doses. The most consistent outcome in the primary literature appears to be impacts on metaphase chromosomes. These outcomes occur at relatively low doses, in intact healthy human cells and across species in cell culture, whole animal and human worker studies. The question remains and this document does not address the underlying mechanism for this outcome. Induction of aneuploidy is another promising mode of action. One mode of action could involve direct damage to the DNA strand resulting in an alteration in chromosome structure or number. This mode would have key events that include: 1) Uptake of Cr(VI), 2) intracellular reduction of Cr(VI), 3) interaction of reductant products with DNA strands, 4) production of chromosomal changes, 5) escape of DNA repair and apoptosis and 6) expansion of damaged cells. Alternatively, the mode of action might not involve direct damage to the DNA. Instead, it could involve direct interactions with the mitotic spindle apparatus and be more of an epigenetic event. This mode would have key events that include: 1) Uptake of Cr(VI), 2) intracellular reduction of Cr(VI), 3) accumulation of intracellular reductant products, 4) interaction of reductant products with mitotic spindle apparatus, perhaps binding to the centrosomes, 5) production of chromosomal changes, 6) bypass of the spindle assembly checkpoint, 7) escape of apoptosis and 8) expansion of damaged cells.
Zhitkovich	Mutagenic mode of action: Hexavalent chromium was overwhelmingly positive for genotoxicity in a large variety of cells and organisms. It was also consistently mutagenic in bacterial and mammalian test systems. The mutagenicity and genotoxicity of Cr-6 result from a direct DNA-damaging mechanism, as evidenced by the induction of mutagenic chromium-DNA adducts and other forms of DNA damage. Formation of chromium-specific DNA lesions at environmentally relevant Cr-6 concentrations and sensitivity of genotoxic responses to manipulations of cellular DNA repair further support the role of direct DNA damage as a primary cause of genotoxicity. Since Cr-6 is taken up via ubiquitously expressed sulfate transporters and its metabolism in cells occur via ubiquitously present ascorbate, glutathione and cysteine, there is no reason to believe that

	the formation of DNA damage in the small intestinal cells and in more extensively studied cell types would be significantly different. Thus, diverse lines of evidence are fully consistent with a mutagenic mode of carcinogenic action for hexavalent chromium. The Draft clearly presented the main arguments for this designation. However, as pointed out above, Tables 4-22 and 4-23 need to be supplemented with information on Cr-DNA adducts. Supporting the importance of Cr-DNA adducts in chromate tumorigenicity are findings from the MOA study by the ACC in which levels of adducts were dramatically higher in the duodenum and jejunum of mice vs. rats. This result mirrors species differences in the intestinal carcinogenesis by chromate and it could not be explained by differences in tissue accumulation of chromium. In contrast to clear positive mutagenicity and genotoxicity data from *in vivo* studies and ascorbate-restored mammalian cell cultures, aneuploidy and epigenetic responses have not yet been tested in animal models and so far have been observed only in ascorbate-deficient cells. In fact, Sun et el. (2009) have found that the induction of epigenetic changes by chromate in human cultured cells occurs only under ascorbate-depleted conditions. The measurements of mutations in KRAS and p53 genes as part of the MOA study sponsored by the American Chemistry Council would not necessarily provide a clear answer about the mutagenic mode of action. A short 3-months duration of this study vs. 2 years for the NTP bioassay certainly diminishes its ability to detect mutations. Among the proposed mutation readouts, three KRAS codons represent a very small and consequently, insensitive mutagenic target. This gene was only rarely mutated in chromate-associated human lung cancers (Ewis et al. 2001). The p53 gene is also uncommonly mutated in cancers among chromate workers (Kondo et al. 1997). The presence or absence of KRAS or p53 mutations do not serve as a strong test for the validity of the mutagenic mode of carcinogenic action, as the frequency of cells with mutated KRAS or p53 can increase through selection of the pre-existing mutant clones whereas transformation process can result from mutagenic events in other components of KRAS and p53 pathways. For example, a large wave of early thyroid cancers among Chernobyl radioiodine-exposed children was caused by translocations in growth factor receptors with almost no RAS and p53 mutations (Nikiforov et al. 1996, Suchy et al. 1998, Williams 2002). <u>Potential alternative modes of action</u>: Two lines of in vivo evidence have been presented to point to a potentially nonmutagenic mode of carcinogenic action. One is based on the drinking water study by DeFlora et al. 2008, which found negative results for DNA damage in the duodenum of mice. However, as discussed in detail above, this study used assays that were insensitive for detection of DNA damage by the employed doses of Cr(VI)in drinking water. Therefore, the negative results of this work were expected and therefore, uninformative. The other observation leading to the discussion of nonmutagenic or indirectly mutagenic mechanisms of carcinogenicity was the presence of diffuse epithelial hyperplasia in the NTP bioassay. Although the NTP study has not found significant necrosis in the small intestine of exposed mice, it is quite possible that the observed hyperplasia was a typical manifestation of regenerative responses. A combination of increased proliferation and

	inflammation could be presented as an alternative mechanism for indirect induction of mutations due to higher rates of cell division and by reactive oxygen species released by the recruited inflammatory cells. This carcinogenic pathway would exhibit a strongly sublinear, threshold-type dose dependence, as it relies on the induction of cell death and small doses would not kill cells. Inflammatory events could also be linked to cell death of chromium-damaged cells, which release pro-inflammatory molecules. The extent of hyperproliferation in chromium-exposed groups was modest, and considering the overall very high rate of cell division in the small intestine, it is hard to see how somewhat faster replication would provide dramatically more spontaneous mutations required for cancer development. At best, the cytotoxicity-induced compensatory proliferation mechanism and the mutagenic mode should co-exist at high tumorigenic doses. The results from the MOA study sponsored by the ACC argue against significant inflammatory responses in the duodenum of chromate-exposed mice, as no increases in the levels of 8-oxodG and in the panel of 22 cytokines have been observed. A statistically significant drop in the ratio of GSH/GSSG was small in its magnitude, further demonstrating that tumorigenic doses were not associated with the state of strongly elevated oxidative stress and inflammation. The presence of chromium-induced hyperplasia could also be viewed as a manifestation of cancer-protective responses by the small intestine. Elimination of genetically damaged cells by apoptosis or another form of cell death is a firmly established protective mechanism against cancer. Thus, there are two opposing interpretations for the toxicological significance of the observed hyperplasia: one is pro-tumorigenic and another is anti-tumorigenic. The supralinear shape of dose-tumor incidence responses in the NTP-2008 studies for female mice is consistent with the engagement of cancer-protective mechanisms. Tumor incidence vs. dose in male mice visually displayed a linear dose-dependence (as shown in Stern 2010). Thus, a hypothetical cytotoxicity-based mechanism with the expected dose-response sublinearity is contradicted by the available evidence.
Zhu	A mutagenic mode of action was proposed as the primary mode of action. On the one hand, EPA discussed data gap and uncertainties about the mutagenic MOA and other possible MOAs. On the other hand, EPA defended the mutagenic MOA despite the lack of data evidence. For example, the only animal study that investigated target tissue genotoxicity (De Flora et al. 2008) reported negative results for DNA-protein crosslinks and DNA adducts in forestomach, glandular stomach, and duodenum of mice exposed to hexavalent chromium in drinking water for 9 month. EPA dismisses the negative finding on the basis of a shorter duration of the study compared with the 2-year NTP study.

B3. A two-year drinking water study in rats and mice (NTP, 2008) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantification is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

Reviewer	Comments
Byczkowski	Apparently, the two-year drinking water study by NTP (2008) is the only, up to date, appropriate cancer bioassay of Cr^{+6} by oral route (this document states - C.f. P. 224, L# 24: "*...No other adequate studies of hexavalent chromium carcinogenicity by ingestion are available...*")
Hamilton	There are no significant concerns about selection of this study, it is clearly the best available study for this type of risk assessment, with the caveats about how these data are interpreted and modeled as discussed elsewhere.
Nordberg	In this document it is not explained to the reader what decided the selection of chromium species and selection of doses and how the chosen doses relate to exposure in drinking water in the general population. Lethal doses should also be given for the chromium species that have been used in the quoted studies. To evaluate and compare the outcome of studies and concentration levels in tissues, a ratio of concentration in dry weight to concentration in wet weight would make it possible and easier to compare reported results and also of possible intake of hexavalent chromium in drinking water. It is told that the animals by increasing exposure to hexavalent chromium in drinking water showed a decrease in intake of drinking water. Influence on different tissues will be found in doing this. On page 112 NTP 2008 decreased body weights could be explained by reduced drinking water consumption.
Patierno	See previous comments above. The NTP study is the best study available but the interpretation of the data and conclusions drawn from it are incorrect. Important supplementary data is preliminarily discussed in the Public Comments.
Rossman	This seems to be the only choice.
Salnikow	Outside of my area of expertise.
Wise	This study is the proper study based on the available data. The study is flawed because only very high doses were considered in the study, thus, there is concern that it may not reflect events at lower doses. The EPA is in the unique position that a study that repeats the one above and extends it to lower doses is almost completed. The EPA should wait for the final results of that study to make the most informed analysis.
Zhitkovich	The selection of a two-year drinking water study in rats and mice by the NTP (2008) for the calculation of an oral slope factor is appropriate. The NTP study was well designed and well executed. No other multiple-dose chronic oral carcinogenicity study in animals is

	available and the dose-dependence from the single ecological study linking chromium-6 in drinking water to human stomach cancers cannot be reliably estimated.
Zhu	The selection of the NTP's 2-year drinking water study in rats and mice (NTP, 2008) is justified. Reasons for why existing epidemiological data were not used for estimating cancer slope factor are acceptable.

B4. The incidence of adenomas and carcinomas combined in the small intestine of male mice from the NTP (2008) two-year drinking water study were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

Reviewer	Comments
Byczkowski	Even though in the NTP (2008) bioassays, only the exposures to the two highest doses of Cr^{+6} in mice and to the highest one dose in rats produced statistically significant carcinogenicity, the selection of this study for derivation of cancer slopes seems to be justified by the description provided in the document. Regarding the (common) practice of combining adenomas and carcinomas in modeling cancer risk, an explanation should be provided that the adenoma is a benign tumor of epithelial tissue with the tendency to become malignant, thus it may - or may not - lead to cancer. Also, the results of NTP (2008) cancer bioassay could be interpreted with the assumption of a threshold.
Hamilton	There is concern about selection of these endpoints to represent cancer risk in these animals as the basis for a human risk assessment. The high doses required to induce these lesions are well above a threshold level that would be of concern in humans under normal exposure scenarios, and these almost certainly represent a scenario where natural reductive defense mechanisms were overwhelmed by the doses of chromium used. Taken together with the more recent 90-day ACC sponsored studies, the MOA for chromium(VI) is most likely a non-mutagenic one involving tissue damage and reproliferation, and would only be seen at doses that are unlikely to ever occur in a human exposure setting, particularly via drinking water. Thus, a threshold based risk assessment is most appropriate, similar to the treatment used for the non-cancer endpoints which are likely to be directly related to the cancer MOA.
Nordberg	See comments above. It should be noted that pH is different in different parts of the gastrointestinal system.

Patierno	See previous comments above. The NTP study is the best study available but the interpretation of the data and conclusions drawn from it are incorrect. Important supplementary data preliminarily discussed in the Public Comments.
Rossman	This seems to be the only choice.
Salnikow	It seems that combining the incidence of adenomas and carcinomas in small intestine was the proper choice for modeling cancer risk. This is supported by the available data and clearly described. The fact that only highest doses produced a carcinogenic effect may indicate high reducing capacity in tested model systems (wild type mice and rats). As suggested in A2 exposing Gulo-/- mice or rats to hexavalent chromium may result in tumor appearance at lower doses. If this will be the case the extrapolation to environmentally relevant doses of chromium exposure will be more feasible.
Wise	If one is going to rely on this NTP study, the selection of the incidence of adenomas and carcinomas in the small intestine of male mice from the NTP (2008) two-year drinking water study to serve as the basis for the quantitative cancer assessment is appropriate. However, scientifically these lesions are not the same and are not necessarily linked. Thus, one or the other should be used.
Zhitkovich	The choice of the combined incidence of adenomas and carcinomas in the small intestine of male mice from the NTP-2008 study for the quantitative cancer assessment was based on a better fit of the multistage model for the male mouse data than for the female mouse data. However, it was unclear why a combination of male and female mouse data sets was not used.
Zhu	For dose-response assessment EPA considered the incidences of adenoma and carcinoma combined in the small intestine of male and female B6C3F mice (Tables 5.3 and 5.4), in the oral cavity (mucosa and tongue) (Tables 5.3 and 5.4) in rats (NTP 2008). (Note the denominators that determine the tumor incidence in small intestine are not consistent with those in Table 4.19). EPA did not consider the incidence of other neoplasm because the incidence is not dose-dependent. The incidence of adenoma or carcinoma in the oral cavity in both male and female rats elevated only at the highest dose, but not at the three lower doses (up to 2.1 and 2.4 mg/kg-d for male and female respectively). To fit a dose-response model to these incidence data that exhibited hockey-sticker shape of dose-response requires a nonlinear (curve-linear) functional form or even a threshold model. Such curve-linear pattern seems inconsistent with the hypothesized genotoxic MOA. The lack of dose-response in these two endpoints was cited as reason for not advancing these two endpoints for final dose-response analysis. Better justification is needed.

B5. The oral slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk of tumors of the small intestine in male mice). Has the modeling been appropriately conducted and clearly described?

Reviewer	Comments
Byczkowski	The oral cancer slope factor was estimated in accordance with the U.S. EPA (2005) guidelines, but to this reviewer the linear cancer extrapolation seems inconsistent with the prior noncancer RfD modeling. While the early preneoplastic epithelial hyperplastic lesions have been modeled as a threshold phenomenon in derivation of RfD, the resultant neoplastic lesions were modeled as a no-threshold linear phenomenon in derivation of cancer slope factors. There seems to be some contradiction, as a threshold-bearing precursor cannot result in the no-threshold adverse effect. In addition, the allometric scaling of cancer slopes, extrapolated from animals to humans based on body weight (to the 3/4 power), seems to be inconsistent with the portal of entry site of action in the GI tract. Typically, this is the local concentration of a chemical at the target site/target tissue that drives an adverse response. Therefore, more appropriate could be a physiologically based pharmacokinetic (PBPK) scaling based on animal-to-human ratio of differences between the rate of absorption and the rate of reduction of Cr^{+6} to Cr^{+3} in gastrointestinal tract contents, as well as the rate of loss of Cr^{+6} from intestinal tract contents to the feces. It seems that appropriately developed PBPK model which recognizes physiological specificity of the different segments of gastrointestinal tract in animals and humans, as presented at the workshop by Summit Toxicology, LLP., may be critical in understanding the mode of action of orally administered Cr^{+6} and in quantitative evaluation of its effects.
Hamilton	As discussed in detail under G1. and G2. responses, this reviewer has considerable concerns about the use of a linear low-dose extrapolation model for assessing chromium cancer risk. The evidence, when objectively assessed, strongly argues for threshold mechanisms both in the gut and systemically, and there is little or no evidence that chromium reaches the systemic circulation as chromium(VI) under exposure scenarios of relevance to human exposures. There is no better candidate for departure from these default EPA assumptions than chromium if EPA is serious about evidence-based risk assessment. Many of the public comments that were available to the reviewers just before and after our May 2011 meeting also raise these issues, and the EPA should, in particular, wait until the recently reported 90-day MOA and PK studies are published and available to them, at which point they should give serious consideration to how these new data inform the likely MOA. Given that most toxicology profiles are only revised every 10-15 years, it is worth waiting for these studies, and taking them as well as the external reviewer comments in mind toward a revised document that will be more accurate and will better stand the test of time. The EPA might also consider asking for a National Research Council Special Emphasis Panel to review all these materials and make a recommendation to EPA regarding chromium(VI) as has been done for other several other key toxicants of concern. In any event the current draft's risk assessment treatment of chromium(VI) is highly flawed and grossly mischaracterizes the likely risk of human

	health effects of chromium(VI) in drinking water based on a careful and thorough assessment of all the available evidence.
Nordberg	The model is clearly described. However I feel very uneasy about extrapolation to lower exposure levels because of chosen exposure doses where LOAEL is identical to the lowest exposure dose administered. Other organizations like IARC do not perform any quantitative evaluation of carcinogenic agents/substances. Threshold concentrations are problematic because of lack of knowledge of how the carcinogenicity develops. Once an organism has been exposed to a substance that can give rise to cancer there is a possibility to such an effect to occur.
Patierno	See previous comments above. The linear extrapolation from the POD is not appropriate. CrVI toxicity and carcinogenicity demonstrates distinct non-linearity and there is little or no relation between what is observed at the highest doses in the NTP study and any physiologically-appropriate or environmentally-relevant exposure.
Rossman	This is outside my field of expertise.
Salnikow	Outside of my area of expertise.
Wise	The modeling has been appropriately conducted and clearly described. More methods are needed to make it clearer. It is possible that Cr(VI) acts via a threshold. The EPA is in the unique position that a study that repeats the NTP study and extends it to lower doses is almost completed. This study will help clarify if there is a threshold. The EPA should wait for the final results of that study to make the most informed analysis.
Zhitkovich	The calculation of the oral slope factor from the POD was appropriately performed. As per US-EPA 2005 Guidelines for Carcinogen Risk Assessment, a linear extrapolation to low doses was used based on the selection of the mutagenic mode of carcinogenic action for chromium-6. The ability of ingested chromium-6 to cause adverse effects at both environmentally relevant and much higher doses has been questioned, given the reported high chromate reducing capacity of the gastric juice and a limited systemic penetration of chromium after oral exposure (as extensively reviewed by the Draft). These considerations led to the formulation of the threshold model of chromium-6 carcinogenesis, which postulates that only doses that exceed the reducing capacity of the tissue (stomach for ingestion exposures) would be carcinogenic (DeFlora 2000). This model would argue that despite the selection of a mutagenic mode of action with the resulting recommendation for default linear extrapolation, the complete detoxification of low-to-moderate chromium-6 doses in the stomach makes it inappropriate to perform linear extrapolation from the POD. The ability of gastric juices to reduce/detoxify chromium-6 is generally accepted in the field; however, studies with human volunteers and other considerations argue against the completeness of the detoxification process. For example, the bioavailability for Cr(VI) was ~10-times higher than for Cr(VI) reduced with orange juice prior to ingestion

	(Kuykendall et al. 1996). The extent of chromium-6 reduction in the stomach is influenced by three factors: its reduction capacity, reduction rate and stomach emptying time. Based on the reported high reduction capacity of the stomach (>80 mg/day, DeFlora et al. 1997), the rate of reduction by gastric juice under fasting conditions could exhibit pseudo-first order kinetics for a broad range of chromium-6 concentrations. This means that the percentage of reduced chromium-6 could be the same for both very small amounts and much larger amounts. Reduction of chromium-6 by artificial gastric juice has been found to follow first order reaction kinetics (Gammelgaard et al. 1999). Consistent with the first-order reaction kinetics, Donaldson and Barreras (1966) found that human subjects excreted in the 24-hr urine 2.1% of ingested 20 ng radioactive chromium-6 whereas Kerger et al. (1997) found that ingestion of 5 mg chromium-6 by human volunteers led to about 1.43% excretion during the first 24 hr (1.43% excretion is a conservative 1/4th estimate from the average 4-day excretion value of 5.7%). Thus, the bioavailability of 20 ng and 5 mg chromium-6 (250,000-fold range) looks quite similar. Donaldson and Barreras (1966) also performed a very important experiment on the bioavailability of 20 ng radioactive chromium-6 that was directly delivered into the duodenum of human subjects. In this case, they found that 10.6% of chromium was excreted in the urine. Since the duodenal delivery represent 100% nonreduced chromium-6, then the amount of nonreduced chromium-6 in their oral route experiment can be estimated from the urinary excretion of 2.1% divided by 0.106 = 19.8%. For the study by Kerger et al (2007), the same type of calculations gives an estimate of 14.3% nonreduced chromium-6 reaching the duodenum. Gammelgaard et al. (1999) calculated a half-life of 23 min for reduction of 0.1mg/L chromium-6 (current MCL for total chromium) by artificial gastric juice. After 1 hr, this reduction rate would leave 16.5% chromium-6.
Zhu	EPA carried out dose-response modeling and BMD estimation for the incidences of adenoma and carcinoma of small intestine in male and female mice separately. EPA stated that it relied on the multi-stage model because the model is preferred by the agency, but gave no justification or explanation. It went on to report an estimated slope of 0.09 (mg/kg-day) and 0.10 (mg/kg-day) for male and female mice respectively. In section 5.3.4 of the Review, EPA reported the CSFs derived on the basis of the cancer incidence of small intestine in male and female mice, and chooses that of male mice because of "the poor fit of the multistage model to the female mouse data". EPA did not provided adequate detail on the modeling efforts, or a discussion and justifications for its final selection (section 5.3.3 and 5.3.4). EPA did provide some detail in Appendix B2, which is essentially the direct output from running the BMDS software, but again no discussion or explanation of the output. It would be helpful and necessary that EPA substantially expand sections 5.3.3 and 5.3.4 to report in greater detail the modeling process, the issues encountered, and justify the decision and choice therein. On a more technical side, examination of Appendix B2 reveals that (1) no standard error is reported for the estimate of model coefficient, and (2) the coefficient of the second order in the polynomial was set to be zero, not estimated. The model did not fit the data at all despite a non-significant p-value for goodness-of-fit test. EPA did not give any explanation or discussion in this regard. The significance of a goodness-fit-test depends on sample size, and a non-significant result does not imply a correct model, especially within a range where there are no data. EPA should explore different options: trying

	different models, considering omitting the highest dose, or considering combine male and female mice. Inclusion of multiple studies, multiple endpoints, multiple model choices, and various BMR levels for deriving a POD is increasingly desirable towards a more systematic and quantitative risk assessment paradigm. It will afford an opportunity to quantify the underlying uncertainties and variations associated with the choices and options made each many stages of the risk assessment process. Within the context of CSF for hexavalent chromium, EPA is in a position to conduct a more thorough and comprehensive assessment by including multiple endpoints, different model forms that allow for nonlinear dose-response, various BMR levels. The outcome will then demonstrate a range for POD and CSF to permit a better quantification and better understanding of uncertainties and variations.

Additional Reviewer Comments

Janusz Z. Byczkowski, DABT, Ph.D.

Additional Comments Submitted by Dr. Janusz Z. Byczkowski

Typos and errors *that should be corrected in the revised version*:

P. 24, L# 4: "...direct measurement of residual Cr(VI) in the *calorimetric* reaction..."
Please change to: "... colorimetric..."

P.26, L# 26: "...Michaelis-Menten uptake kinetics..."
Please change to: "...Michaelis-Menten-type uptake kinetics..."

P. 51, Figure 3-6, it would help the reader if non-standard abbreviations were explained in the figure legend - e.g., *DSB*, *SSB*, etc.

P. 52, L# 38: "...to describe kinetics *of* humans..."
Please change to: "...kinetics in humans..."

P. 84, L# 36: "...*colestasis*, as other markers of *colestasis*..."
Please change to: "...cholestasis..."

P. 229, L# 33: "...(see Section *4.6.3.4*)..."
Such a section does not exist in the reviewed document.

P. 244, L# 7: "...*Bukowksi*, JA..."
Please change to: "...Bukowski..."

Additional Comments Submitted by Dr. Konstantin Salnikow

Errata:

Regarding the form of the draft it should be noted that, although this is not a manuscript, it is necessary to correct errors and mistakes in the draft content.

Below are several examples:

1. **$K_2Cr_2O_4$** – does not exist (page 30, table 3-7; page 45), **should it be K_2CrO_4.**
2. Table 2-1, page 6 – **Cr_2O_3** is chromium (III), not hexavalent chromium. **$BaCrO_4$** is barium chromate, not barium oxide.
3. Table 2-5, page 18 – "accumulates Cr(V)", the intent of this statement is not clear because this form is short living and unlikely that is can accumulate.
4. Page 35 and Table 3-9, **K_2CrO_7** - does not exist.
5. None existing or wrong citations:

Kumulainen, 1991 (page 7), **should be Kumpulainen, 1992** (page 251).

Salnikov and Zhitkovich, 2009 (page 50); Salnikov and Zhitkovich, 2008 (page 257), **should be Salnikow and Zhitkovich, 2008**.

Costa and Klein, 2004 (page 66); Costa, M.; Klein, C.B. 2008 (page 245), **should be Costa and Klein, 2006**.

LeVina et al., 2003, 2007, (page 50) **should be Levina et al., 2003, 2007**.

Kasprzak, 1996 (page 189), **no reference provided.**

Campbell J.L.; Tan,Y.; Clewell, H.J. (2009) Development of a PBPK model for hexavalent chromium in rats and mice to estimate exposure to oral mucosa and small intestine. Toxicologist 108(1):98 (Abstract) Poster ID # 108. This is a **questionable citation**.

Sun et al. (2009), (page 188), **no reference provided.**

Davies, JM. (1979) Lung cancer mortality of workers in chromate pigment manufacture: An epidemiological survey. J Oil Chem Assoc 62:157-163, (page 245), **should be: J Oil Colour Chem Assoc 62:157-163**.

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Additional Comments Submitted by Dr. Anatoly Zhitkovich

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Appendix A: Individual Reviewer Comments

POST-MEETING COMMENTS SUBMITTED BY

Janusz Z. Byczkowski, DABT, Ph.D.

Janusz Z. Byczkowski, DABT, Ph.D.

Contract No. EP-C-07-024
Task Order No. 92
March 3, 2011

Toxicological Review of Hexavalent Chromium

Post-meeting

Answers to General Questions

G1.

Logical - yes; clear - mostly; concise - no, as there is a very large body of information covered by this Toxicological Review.

While the scientific evidence of both noncancer and cancer hazards of oral exposures to Cr^{+6} has been appropriately reviewed and synthesized, the conclusions and numerical derivation of toxicity values are mostly based on strict (default) interpretation and literal application of U.S EPA guidelines, rather than on the current scientific understanding of the mode of action of Cr^{+6}.

G2.

It seems that up to date (around the year 2009), all important studies (and some unimportant too) have been already covered by this Toxicological Review. However, I strongly suggest that the results of study presented at the workshop by ToxStrategies, Inc., and especially the physiologically based pharmacokinetic (PBPK) modeling by Summit Toxicology, LLP., should be included in the revised Toxicological Review of Hexavalent Chromium document.

Answers to Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Hexavalent Chromium

A1.

The two-year drinking water study by NTP (2008) seems to be the most comprehensive from all available chronic bioassays, and thus, it is suitable as the basis for derivation of the RfD.

However, there is still some concern regarding the selection of the NTP study for developing of RfD (this document states - C.f. P. 85, L# 1: "*...Urinalysis showed dose-related decreased volume and increased specific gravity, consistent with decreased water intake. NTP (2007) suggested that decreased water intake was due to decreased palatability...*" and then, P.89. L# 34: "*...Drinking water consumption was reduced...*") Thus, the reduced drinking water consumption, and consequently at least partial dehydration, may have increased the osmolality of gastrointestinal fluid, which could be a significant confounder in the chronic

toxicity study, even though (according to the quotation on P. 120) the Technical Report by NTP (2008) attempted to dismiss such a concern.

A2.

Diffuse hyperplasia, in itself, is considered to be a physiological (normal) response to several stimuli, as the cells of a hyperplastic growth remain sensitive to normal regulatory control mechanisms. Such a physiological proliferation of cells may be secondary to several pathological factors (e.g., the increased osmolality, changed pH, infection by Helicobacter, etc). Still, the proliferation in "diffuse hyperplasia" is a normal process - although, it may be generated in response to abnormal condition (in contrast to neoplasia, where the proliferation in itself becomes abnormal). On the other hand, the hyperplasia is a common early preneoplastic response to potentially carcinogenic stimuli.

Considering its rather ubiquitous and nonspecific character, it is remarkable that even no single case of epithelial hyperplasia was found during the two-year study by NTP (2008) in duodena of as many as 100 male and female control mice. Taking this result at a face value, it may be assumed that the diffuse epithelial hyperplasia in duodena could be an early biomarker of oral exposure to Cr^{+6}, at least in the two-year NTP (2008) bioassay study.

However, another end point - the pathological changes in the liver were noted in the same study with significantly increased frequency at the lowest dose employed. Even though they may be considered somewhat less "specific" than the epithelial hyperplasia (because they were observed at low frequency also in controls), but perhaps they could be more relevant to the systemic toxicodynamics of Cr^{+6} - appearing further away from the portal of entry then the hyperplasia and apparently being less sensitive to the potentially confounding effects of the reduced drinking water consumption.

A3.

The benchmark dose modeling was applied in accordance with the *U.S. EPA Benchmark Dose Technical Guidance Document (2000)* in the prescribed manner. However, the modeled BMD, derived from the diffuse epithelial hyperplasia, was supported only by the three dose-points (including zero dose). In contrast, the pathological changes in the liver were consistently fitted, for both female rats and mice with Log-logistic model which included all the five data points (including controls), and thus, they could be considered to be robust dose-response adverse effects for derivation of BMD. So, the pathological changes in the liver may be used as alternative end points for derivation of BMD.

A4.

While the uncertainty factors (UFs) were applied in accordance with the U.S. EPA guidelines, the use of the UF = 10 for extrapolating the toxicity value based on effects in the portal of entry of Cr^{+6} in animals to

predict GI effects in humans, seems to be problematic. Thus, both the reductive capacity of human gastrointestinal fluids and the antioxidant protection of human tissues exceed those in mice. So, adequately nourished and hydrated humans should be less vulnerable than mice to the adverse GI effects of oral exposure to Cr^{+6}, rather then 10 times more sensitive, as the U_A=10 may suggest.

(B) Carcinogenicity of Hexavalent Chromium

B1.

Even though the U.S. EPA (2005) guidelines were applied appropriately, there is no direct evidence of dose-dependent carcinogenicity by orally administered Cr^{+6} in humans (C.f. P. 236, L# 33: "*...EPA concluded that the exposure-response analyses presented by Zhang and Li (1997), Beaumont et al. (2008), and Kerger et al. (2009) are not based on the quality of data that is needed to support a conclusion regarding the presence or absence of a dose-response among the observed cancer rates in these villages. The other epidemiologic studies did not find a significant correlation between hexavalent chromium concentrations in drinking water (or proximity to the source of hexavalent chromium soil contamination) and cancer...*")

The classification of hexavalent chromium as "*likely to be carcinogenic to humans by the oral route of exposure*" is based on carcinogeniesis observed only at the highest dose levels employed in animal studies. Therefore, it may, or may not be relevant to humans at environmentally relevant exposure concentrations.

B2.

The positive laboratory results of mutagenicity tests do not prove genotoxicity and are not necessarily biologically relevant to humans exposed *in vivo* to the environmentally relevant concentrations of Cr^{+6}.The mutagenicity results from humans occupationally exposed to Cr^{+6} (Table 4-25) were inconsistent (this document states - C.f. P. 178, L# 14: "*...In general, associations between hexavalent chromium exposure and mutagenicity in workers are uncertain...*") and actually, the results presented in the document did not prove the direct genotoxic mode of action of Cr^{+6} *in vivo*.

An alternative, indirect mode of action seems plausible to this reviewer. As discussed in Section 4.5.2., the intracellular products of one-electron reduction of Cr^{+6} *in vivo*, causing oxidative stress and free-radical damage to cellular macromolecules, seems to be responsible for initiating carcinogenicity at relatively low dose levels. Erosion of GI tract mucosa with inflammation that followed at high Cr^{+6} dosage (discussed in section 4.2.1) apparently caused promotion in the lesions, which eventually progressed to benign and malignant tumors. Importantly, such a mode of action implies a threshold phenomenon, *i.a.* due to antioxidant protection of cells.

Janusz Z. Byczkowski, DABT, Ph.D.

B3.

Apparently, the two-year drinking water study by NTP (2008) is the only, up to date, appropriate cancer bioassay of Cr^{+6} by oral route (this document states - C.f. P. 224, L# 24: "*...No other adequate studies of hexavalent chromium carcinogenicity by ingestion are available...*")

B4.

Even though in the NTP (2008) bioassays, only the exposures to the two highest doses of Cr^{+6} in mice and to the highest one dose in rats produced statistically significant carcinogenicity, the selection of this study for derivation of cancer slopes seems to be justified by the description provided in the document.
Regarding the (common) practice of combining adenomas and carcinomas in modeling cancer risk, an explanation should be provided that the adenoma is a benign tumor of epithelial tissue with the tendency to become malignant, thus it may - or may not - lead to cancer.
Also, the results of NTP (2008) cancer bioassay could be interpreted with the assumption of a threshold.

B5.

The oral cancer slope factor was estimated in accordance with the U.S. EPA (2005) guidelines, but to this reviewer the linear cancer extrapolation seems inconsistent with the prior noncancer RfD modeling. While the early preneoplastic epithelial hyperplastic lesions have been modeled as a threshold phenomenon in derivation of RfD, the resultant neoplastic lesions were modeled as a no-threshold linear phenomenon in derivation of cancer slope factors. There seems to be some contradiction, as a threshold-bearing precursor cannot result in the no-threshold adverse effect.
In addition, the allometric scaling of cancer slopes, extrapolated from animals to humans based on body weight (to the 3/4 power), seems to be inconsistent with the portal of entry site of action in the GI tract. Typically, this is the local concentration of a chemical at the target site/target tissue that drives an adverse response. Therefore, more appropriate could be a physiologically based pharmacokinetic (PBPK) scaling based on animal-to-human ratio of differences between the rate of absorption and the rate of reduction of Cr^{+6} to Cr^{+3} in gastrointestinal tract contents, as well as the rate of loss of Cr^{+6} from intestinal tract contents to the feces.
It seems that appropriately developed PBPK model which recognizes physiological specificity of the different segments of gastrointestinal tract in animals and humans, as presented at the workshop by Summit Toxicology, LLP., may be critical in understanding the mode of action of orally administered Cr^{+6} and in quantitative evaluation of its effects.

Additional Comments:

Typos and errors *that should be corrected in the revised version*:

P. 24, L# 4: "...direct measurement of residual Cr(VI) in the *calorimetric* reaction..."
Please change to: "... colorimetric..."
P.26, L# 26: "...Michaelis-Menten uptake kinetics..."
Please change to: "...Michaelis-Menten-type uptake kinetics..."

P. 51, Figure 3-6, it would help the reader if non-standard abbreviations were explained in the figure legend - e.g., *DSB*, *SSB*, etc.

P. 52, L# 38: "...to describe kinetics *of* humans..."
Please change to: "...kinetics in humans..."

P. 84, L# 36: "...*colestasis*, as other markers of *colestasis*..."
Please change to: "...cholestasis..."

P. 229, L# 33: "...(see Section *4.6.3.4*)..."
Such a section does not exist in the reviewed document.

P. 244, L# 7: "...*Bukowksi*, JA..."
Please change to: "...Bukowski..."

POST-MEETING COMMENTS SUBMITTED BY

Joshua W. Hamilton, Ph.D.

Post-Meeting Reviewer Comments on
EPA Draft Toxicological Review of Hexavalent Chromium
(dated September 2010)

Joshua W. Hamilton Ph.D. 6/12/2011
Senior Scientist, Bay Paul Center, Marine Biological Laboratory
Professor (MBL), Pathology and Laboratory Medicine, Brown University

General Charge Questions:

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for non-cancer and cancer hazard?

G2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

Reviewer Response:

In general, the report is a concise presentation of the vast primary literature for chromium toxicology and carcinogenicity and the writing is generally clear. However, there are specific sections with deficiencies as noted below in detail, and many other sections and specific comments that are not logical. In many cases a statement in one section is either not a logical extension of the data presented, or is in opposition to a statement elsewhere. Overall, the greatest concern is with the logic regarding the choice of a mode of action (MOA), which is the basis for many of the subsequent assumptions that are made, the default values that are chosen, and risk assessment modeling that follows from these choices. In this reviewer's strong opinion – and in the consensus opinion of the external reviewers who are experts in this area and who discussed this at the May 12, 2011 meeting – Cr(VI) is highly unlikely to act via a mutagenic mode of action in vivo. Rather, a careful review of existing information, as well as emerging studies all strongly indicate that the likely MOA involves a threshold mechanism that supports both the physiological uptake-reduction model of DeFlora and the cellular uptake-reduction model of Wetterhahn that were previously proposed. The current EPA draft document concludes that chromium(VI) acts via a mutagenic MOA by all routes of exposure, a conclusion that is illogical given the current state of knowledge of chromium biology and toxicology as already presented in this draft report, and also based on the recently emerging data from a series of 90-day rodent MOA studies sponsored by the American Chemistry Council (ACC). This is the most important and central point since the choice of a mutagenic MOA then drives all other considerations in this document. Specific areas of concern are outlined by chapter and section below in detail in a combined response to G1. and G2.

Joshua W. Hamilton, Ph.D.

Chapter 1

EPA should include more definitive information about the literature it reviewed that contributed to this draft report. It currently states that the relevant literature was reviewed through September 2010 but the external reviewers all noted at the May 12 meeting a number of gaps in the literature being cited in the current draft. In addition, it would be helpful to know how many studies the EPA identified as being part of the chromium literature, how many it reviewed, how many it set aside or did not review, what criteria were used to include or exclude a study, etc. For example, a statement such as: *"The EPA identified 26,839 peer-reviewed scientific publications in PubMed from 1950 through September 2010 using the keyword 'chromium.' Of these, 9,456 were determined to be relevant to the current draft based on the criteria of covering aspects of chromium biology, toxicology, environmental chemistry and epidemiology."*

Chapter 2

On page 14 the draft states that,*"Natural occurrence of hexavalent chromium is rare ..."* This statement should be qualified since geochemical surveys by the U.S. Geological Survey and others have indicated that there are background levels of naturally occurring Cr(VI) in most groundwater, and that these levels are typically in the range of 2-5 ppb but can be up to 3-5 times higher than that in certain areas. Likewise recent reports of Cr(VI) levels in soil and house dust indicate that there are natural sources, or at least sources that cannot be identified as being specifically anthropogenic, that contribute to the background levels and background exposures of people throughout the U.S. A more detailed literature review and discussion regarding background levels of Cr(VI) in soil, air and water, including more up-to-date information on such levels, should be included in this chapter to provide context for subsequent discussions regarding human exposure through drinking water. Keep in mind that, until recently, analytical methods to detect total chromium and to speciate chromium(VI) accurately were difficult, with higher detection levels than many natural sources contain. Further, speciation of chromium(VI) at very low levels has been very problematic until recently, so much of the older environmental data are inaccurate or had non-detect values where reanalysis has revealed widespread chromium(VI) background levels in the environment. This is of importance not only for understanding the potential contribution of these background levels to total chromium exposure but also in setting practical regulatory levels. Clearly it is of little value for the EPA to calculate a maximum contaminant level (MCL) or public health goal (PHG) that is lower that typical background levels since this would be virtually impossible to achieve by most public drinking water systems with limited resources, and because such background levels are unlikely to represent a significant health risk (see discussion below).

Chapter 3

This is an important chapter that would greatly benefit from reorganization and from synthesis of information. A number of studies are described here sequentially, and for several of these the draft document speculates on possible mechanisms. However, the order of presentation is not logical, and there is little in the way of more global synthesis of results and the conclusions that can be drawn – or those areas that remain controversial or poorly understood – while there are several areas where a statement in one section is contradicted by a statement elsewhere. Because the toxicokinetics of chromium is so central to its biology and toxicology, this is a critical chapter for its overall evaluation and therefore this chapter should be revised.

Regarding organization, Section 3.3 is extremely important for understanding the studies described throughout the remainder of the chapter, providing context for extracellular versus intracellular reductive metabolism. It is suggested that this section should be moved to just before or immediately after the current Section 3.1. Likewise, Section 3.6 on Cr(III) and its nutritional benefit and essentiality is extremely important for understanding how the body normally absorbs, distributes and excretes chromium. From the standpoint of human environmental exposures, Cr(VI) is primarily, but not exclusively an anthropogenically derived form of chromium that is principally encountered and used in occupational settings, but humans and all other life forms have been dealing, both physiologically and biochemically, with Cr(III) and reduction of background levels of Cr(VI) for the entire history of life on Earth. It is recommended that Section 3.6 should be moved to the beginning of this chapter and greatly expanded. The current section on this important topic is extremely short, superficial, and inaccurately presents this subject as an area of controversy in the field.

Cr(III) nutritional biochemistry has been extensively studied over the past fifty-plus years with a large and robust literature. Only two toxicologists are cited as the sources of the "current debate" about this, whose individual views have been well aired in their review articles but which represent views that are generally considered to be well outside the mainstream of toxicology and nutritional biochemistry. Citing their views so prominently in this short section does not balance well with the wealth of studies and numerous other investigators over decades who have concluded that there is a beneficial role of Cr(III) in human and animal nutrition and have demonstrated an underlying biochemistry and physiology that supports this role. This section should be greatly expanded and treated in a more balanced fashion since it sets the stage for understanding how Cr(III) is treated by the body in all aspects of toxicokinetics, and therefore also provides valuable insight into Cr(VI) toxicokinetics that largely explains much of the experimental literature on chromium disposition in intact animals and humans as discussed in more detail below. See for example reviews by W Mertz (J Nutr 1993, 123:626-633; Nutr Rev 1995, 53:179-185), RA Anderson (Reg Tox Pharm 1997, 26:S35-41), HC Lukaski (Ann Rev Nutr 1999, 19:279-302) and JB Vincent (J Am College Nutr 1999, 18:6-12) for summaries of this earlier literature.

Some of this information is alluded to – for example, the end of the primary paragraph on page 44 beginning with *"Aitio et al. (1988) developed ..."*, which represents an extremely important body of literature

on Cr(III) toxicokinetics and which should be moved forward with section 3.6 and greatly expanded by citing other relevant literature. There are dozens of studies of Cr(III) uptake and kinetics. The current chapter leaves the impression that Cr(III) gastrointestinal (GI) uptake is uniformly low, and that chromium is not normally found above background levels in urine, therefore if one administers Cr(VI) and sees a dose-dependent increase in blood, urinary or tissue levels it is evidence of Cr(VI) uptake as Cr(VI). But there are a number of uptake studies of chromium picolinate and other natural and man-made Cr(III) complexes, as well as Cr(III) uptake in chromium-sufficient versus chromium-deficient diets, that defy this simple interpretation. For example, one study described on page 45 by Kerger et al. (1997) reported that *"ingestion of chromium picolinate resulted in significantly elevated urine concentrations such that participants routinely exceeded background."* Similar elevations have been reported for chromium picolinate in the nutritional literature. Likewise, other studies have shown an inverse correlation between Cr(III) levels in diet and the uptake, distribution, tissue storage and excretion of Cr(III), indicating that the body tightly regulates Cr(III) kinetics to maintain a steady-state body burden and availability of the nutritionally active form of Cr(III), and can actively take up Cr(III) from the GI when the body senses that it is deficient, or decrease uptake and increase excretion when internal stores are sufficient or exceeded.

A related and key concept that is alluded to in this section but never addressed directly is the behavior of chromium in serum and red blood cells (RBC). It has been well established, beginning with RBC labeling studies going back to the 1950's in which RBC are incubated with Cr(VI) ex vivo, that Cr(VI) is readily taken up by RBC, rapidly reduced, and in the process forms highly stable chromium adducts on hemoglobin and other macromolecules which are very long lived, essentially remaining intact for the lifetime of the cell. In this way the half-life of RBC in humans and experimental animals was established (with human RBCs having a half-life of ca. 110-120 days) and this tool has also been used to look at RBC turnover. This is alluded to on page 27, where it states that *"The partitioning of hexavalent chromium from plasma into erythrocytes is significant. It has been used as a biomonitoring endpoint ... and is responsible for the observed residence time of chromium in whole blood ..."* Conversely, in the human and animal studies that were described in Chapter 3, it has been consistently shown that when Cr(VI) is administered orally or by gavage, there is a transient increase in both serum and RBC levels which rapidly return to baseline. Thus, it is highly unlikely that the chromium in the blood of these animals and humans was Cr(VI), or else the RBC would have been stably labeled. It was noted on page 45, for example, in describing the human studies of Paustenbach et al. (1996) where there was oral exposure to Cr(VI) that *"both plasma and RBC chromium concentrations returned rapidly to background levels within a few days, again suggesting that concentrations of 10 mg Cr(VI)/L or less in drinking water of humans appears to be completely reduced to Cr(III) prior to systemic distribution."* This is an extremely important experimental observation in humans – and one of the most important statements in this draft document -- that directly addresses the issue of whether Cr(VI) is taken up by the human GI as Cr(VI) and whether it survives as Cr(VI) in the circulation.

Similar results were seen in the Sutherland et al. (2000) rat study in which the blood kinetics, and lack of chromium increases in brain or other distal tissues argued strongly that it was Cr(III) rather than Cr(VI) that was taken up by the gut. Were this not the case, they should have observed stable chromium labeling of the RBC and elevated chromium levels in distal tissues. But they reported the opposite, and this also strongly indicates in this key rodent study that Cr(VI) failed to survive as Cr(VI) in crossing into the bloodstream from a GI exposure. And elsewhere in Chapter 3, similar results are reported that would lead to the same conclusion, yet the text alludes to transient RBC uptake as possible evidence that Cr(VI) is being taken up from the GI tract as Cr(VI). This is a highly flawed, illogical argument that appears throughout this document. Likewise it is now well known that there are specific uptake, transport and storage mechanisms for nutritionally active Cr(III) that must be taken into account in any measurements of chromium in the blood or other tissues. In fact, none of the studies presented in Chapter 3 provide any direct evidence that any Cr(VI escapes the GI tract as Cr(VI) except perhaps where normal gastric reduction is bypassed or the bolus doses are so large as to completely overwhelm the reductive capacity of the gut, which is likely what occurred in the NTP (2008) study. This should be more clearly described in this chapter, perhaps with a new section prior to current section 3.5 that presents the theoretical PBPK model since this model also critically relies on how one interprets the in vivo data.

Specific edits suggested below are based on these concerns (changes underlined):

- Page 24, Line 24 – *"...for the chromium administered as hexavalent ..."*
- Page 25, Line 7 – *"...of the chromium administered as hexavalent ..."*
- Page 26, Line 3 – *".... Generally, absorbed chromium is ..."*
- Page 26, Line 9 – *"... toxicokinetics of chromium, ..."*
- Page 26, Line 16 – *"... comparing administration of Cr(III) ..."*
- Page 30, Line 2 – *"... bioavailability of chromium administered as ..."*
- Page 35, Lines 7-11 – two sentences beginning with *"The reason for the higher ..."* This is highly speculative and should be deleted. This could all be Cr(III) rather than Cr(VI) using the arguments above.
- Page 36, Lines 25-28 – end of sentence beginning with *"... indicating that a portion of the Cr(VI) escaped extracellular reduction ..."* This is also highly speculative and is actually counter to the data presented, which clearly show transient blood levels that are indicative of Cr(III) distribution, not Cr(VI), as more appropriately alluded to in Lines 32-34 where is says *"Brain, ovarian, and whole-blood concentrations were below detection limits in all exposed groups. The lack of concentrations in whole-blood was attributed to rapid delivery of Cr to tissues and clearance of plasma Cr."* The lack of stable blood chromium clearly indicates that it could not have been absorbed as Cr(VI) or else the RBC would have shown significant and stable elevations.
- Page 38, Line 13 – *"... did not alter GI uptake appreciably at these concentrations."*

- Page 41, Line 1 – *"Chromium is capable of crossing the placenta."*
- Page 41, Line 15-17 – *"Absorption and elimination of chromium was evaluated .... Following ingestion by human volunteers of either trivalent or hexavalent chromium in single or multiple drinking water doses."*
- Page 42, Lines 23-26 – This statement beginning with *"Because the Cr(VI) increases ..."* is incorrect. Increases in RBC chromium that are stable over time, with the same half-life as the RBC, would be indicative of uptake of chromium as Cr(VI) by the RBC, but if the increase in RBC chromium is transient, as in this case, then it cannot be due to Cr(VI) in the blood but is most likely a Cr(III) complex that is carried by the RBC but not covalently bound. Evidence for this is cited elsewhere in this chapter (see middle of page 44 and top of page 46, for example) and in the broader Cr(III) literature. The draft report is illogical since it currently argues this issue both ways depending on the study being discussed.
- Page 42, Lines 30-35 – These two sentences beginning with *"The higher bioavailability ..."* are highly speculative and well outside the boundaries of the actual data presented and discussed above. If the report is going to speculate, it should pull all this into a separate section and synthesize it across all the studies that were cited. Otherwise delete these speculations since they do not have a factual foundation.
- Page 49, last line – *" ... chromium administered as Cr(VI) distributes ..."*
- Page 58, Lines 2-3 – *" ... greater percentage of chromium administered as Cr(VI) than Cr(III) is absorbed."* Delete sentence reading *"This implies that some Cr(VI) escaped reduction ..."* since this is not implied by the actual experimental data based on the above arguments.

Taken together, these studies, as argued above, lead one to only one logical conclusion: Cr(VI) is not taken up by the gut as Cr(VI), nor does it survive as Cr(VI) in the systemic circulation in these studies. It is only by greatly exceeding the normal doses and reductive capacity of the gut that one can see any signs of Cr(VI) surviving to reach other tissues. Therefore there is a clear biochemical barrier, a threshold, to Cr(VI) uptake and systemic exposure under normal physiological conditions. This must clearly be taken into account in any analysis of MOA and resulting risk assessment for this toxicant.

Chapter 4

Liaoning Province, China studies – At the beginning of this chapter (Pages 68-76) the EPA presents an extensive review and discussion of the various reports related to the study of populations in China near a site of chromium-contaminated drinking water from a nearby industry. Depending on the authors and methods used – particularly certain assumptions regarding age adjustment, use of an urban area as a control population, and exposure estimates -- the reports of this data set either find no statistical association between stomach cancer incidence or a modest elevation. The other epidemiology studies cited in this chapter report

no statistical correlation between drinking water chromium exposure and cancer incidence. It is of considerable concern to this reviewer that the EPA has chosen in some places to highlight this one positive report and elevate it to the level of their major recommendations, such as on Page 239 of Chapter 6 (Major Conclusions) where they state that there is *"evidence of an association between oral exposure to hexavalent chromium and stomach cancer in humans."* Yet elsewhere, they state, appropriately, that *"this risk has not been established in other populations exposed to drinking water contaminated with hexavalent chromium. The epidemiology data are not sufficient to establish a causal association between exposure to hexavalent chromium by ingestion and cancer."* (Page 201, Lines 20-23; and similar language on Page 205, Lines 33-36)

It is inappropriate to "cherry-pick" a single study – indeed, a single treatment among three treatments of the same study – if this is going to influence their major recommendations regarding the human carcinogenicity of Cr(VI) via ingestion. Many of my other comments and edits relate to this concern, as well as previous concerns regarding the toxicokinetics of Cr(VI) via ingestion and the misinterpretation of those results with respect to mode of action and carcinogenic potential in animals and humans as detailed below. This reviewer feels strongly that the Liaoning Province study and its various treatments should be set aside, since the initial study (first reported in 1987) is highly flawed by today's epidemiology standards, there is a lack of information that would allow others to reevaluate this study in the manner that would most directly address the potential correlation, and the three subsequent treatments by original authors Zhang and Li (1997), by Beaumont et al. (2008) and by Kerger et al. (2009) -- each of which makes slightly different assumptions in order to fill critical data gaps -- reached different conclusions. It is also important to point out that this population was exposed not just to extremely high levels of Cr(VI) but also to industrial effluent which contained high levels of a number of other chemicals of concern including sulfates, acids and other toxicants. Thus, even if an association was found, it is not possible to attribute this to Cr(VI) per se and could be the result of either other contaminants or a statistical anomaly based on the high rates of stomach cancer in China which are diet- and province-related. For these reasons this study and its series of treatments cannot be the basis for risk characterization or risk assessment, and should be set aside.

Section 4.4 also requires extensive revision and expansion. The first paragraph reports only briefly on the extensive literature examining occupational exposures and cancer, principally lung cancer. This section, and this literature, should receive a much more extensive treatment since these studies are our best data regarding human exposures to Cr(VI). It should be noted that in addition to inhaling extremely high levels of Cr(VI) prior to the advent of industrial hygiene practices in the 1960's and 1970's (levels as high as several mg chromium(VI) per cubic meter) these workers had extensive dermal exposures, and also had extensive ingestion exposure since it is well known that individuals exposed to high levels of dust swallow a large fraction of what they inhale either through direct deposition in the mouth, nose and throat, and via mucocilliary clearance of the pulmonary system. Thus, these occupational epidemiology studies are

extremely important in understanding the toxicology of chromium(VI). Several key points will be made below citing a few critical studies, but the EPA should more thoroughly review this entire literature.

The U.S EPA's current risk assessment for chromium(VI) via inhalation is based principally on the studies by Mancuso of older worker populations through the 1960's prior to the advent of modern industrial hygiene practices. Similar risks were observed in other occupational studies of workers from the 1940's through 1970's who were exposed to much higher levels of chromium than are encountered in occupational settings today, such as those by RB Hayes et al. (Intl J Epi 1979, 8:365-374; Am J Indust Med 1989, 16:127-133), JM Davies (Br J Indust Med 1984, 41:158-168), T Sorahan et al. (Br J Indust Med 1987, 44:250-258), and R Kishi et al. (Am J Indust Med 1987, 11:67-74). Subsequent epidemiology studies of workers exposed to lower levels of chromium since the 1960's have not only provided estimates of risk at these high doses, but have also provided important information as to lower occupational levels at which no increases in lung cancer or other health effects were observed. These more modern studies have also taken into account other factors, such as accounting for cigarette smoking and other confounding variables, whereas many of the older studies did not control for these important confounders (see for example, KD Rosenman, Am J Indust Med 1996, 29:491-500). It should be noted that, where it was reported, virtually all the lung cancer cases in these occupational studies occurred in smokers (see for example Pastides et al., Am J Indust Med 1994, 25:663-675; T-C Aw, Reg Tox Pharm 1997, 26:S8-12). These studies not only provide better estimates of the actual health risks attributable to occupational chromium exposure, but also an estimate of a practical threshold below which we would predict either no effects, or risk of effects that are so low that it cannot readily be detected even in large populations of exposed people.

Gibb et al. performed a follow-up study (Am J Indust Med 2000, 38:115-126) of a worker population in Baltimore MD that had previously been studied by Hayes et al. (Int J Epi 1979, 8:365-374) in examining the relationship between chromium(VI) exposure and cancer incidence. In the Gibb study, the workers were stratified according to different levels of cumulative exposure to chromium, allowing a more detailed examination of the potential dose-response. Cumulative exposure was expressed as $\mu g/m^3$-years (1,000 ng/m^3-years), integrating both the chromium level and the total time of exposure at that level. This is similar to smoking data that express cumulative dose as "pack-years" and is based on the observation that the risk of a 40 pack-year smoker who used 1 pack per day for 40 years is similar to that of a 40 pack-year smoker who used 2 packs per day for 20 years. In the Gibb study, the lowest quartile of workers had exposure to chromium between 0 and 1.5 $\mu g/m^3$-years (1,500 ng/m^3-years). This group had an observed/expected lung cancer ratio of 0.96, i.e., it was slightly less than expected from the comparison population (the general population of Maryland) that had no occupational chromium exposure.

Pastides et al. examined a group of chromate production company workers in North Carolina (cited above), focusing on the possible differences in risk between cohorts of workers who were exposed to chromium under the older conditions and processes of the 1940's through 1960's and those who began work

after 1971 in a modernized factory in which both the chemical process and the exposure levels to chromium had been modified. They found a slightly increased risk of lung cancer, proportional to exposure, in the older cohort working under the higher dose exposure conditions, as had been reported previously in other studies. However, the workers in the modernized factory had no excess of lung cancer, all cancers, heart disease, or all causes of death over an 18 year period. Personal monitors for the workers indicated that the chromium(VI) levels were all below 50,000 ng/m^3, and most were below 25,000 ng/m^3, with the majority in the range of 500-10,000 ng/m^3. Average duration of employment was 9.5 years, such that cumulative dose would have averaged 4,750-95,000 ng/m^3-years. Dividing the workers into two groups of exposure, i.e., those working less than 10 years versus those working more than 10 years, indicated no difference in mortality, further suggesting that these workers had no significant increase in cancer or other health risks from either the higher or lower chromium exposures. Similarly, Aw reported (cited above) that workers in the more modernized plants who were occupationally exposed to chromium since the 1960's showed no increase in disease risk, as was also noted by S Langard et al. (Br J Indust Med 1990, 47:14-19).

WJ Blot et al. performed a large and comprehensive study (J Occup Environ Med 2000, 42:194-199) of a group of 51,899 workers of the Pacific Gas & Electric (PG&E) Company. A sub-set of 3,796 these workers had been exposed occupationally to chromium(VI), either as gas generator workers or trainees at the Kettleman CA station which used chromium as a rust inhibitor in cooling tower water at PG&E natural gas transfer stations from the 1950's through the 1980's. Examination of these workers for specific cancers, all cancers, specific non-cancer diseases, and all diseases indicated no increased incidence in any adverse health outcomes in relation to chromium exposure. In fact, the total cancer and lung cancer standardized mortality ratios (SMRs, observed/expected ratios) were 0.64 and 0.81, and 0.55 and 0.57, respectively, for these two groups of chromium-exposed workers, which was less than those of the overall PG&E worker group and substantially less than those of the general California population against which they were compared. SMRs for all causes of death were also low (0.79 and 0.68, respectively). Likewise, JD Boice et al. performed a large and comprehensive study (Occup Environ Med 1999, 56:581-597) of a group of 77,965 workers at an aircraft manufacturing plant in California. A sub-set of 3,634 of these workers were exposed to chromates and other chemicals as part of airplane production for a total of 88,224 person-years of exposure and a mean of 24 working years per person of exposure. The SMR for total cancers was 0.93, and the SMR for lung cancer was 1.02. As with the Blot study, there was no association of any adverse cancer or non-cancer health outcome with chromium exposure in this group, nor did the overall worker population have an increase in overall or specific mortalities as compared to the general population despite exposure to a number of occupationally related chemicals.

Taken together, these occupational studies indicate that, although previous historical exposure conditions were associated with a modest risk of lung and respiratory cancer (average of 2- to 4- fold increased lifetime risk, as compared, for example, to a 10- to 20-fold increased risk for cigarette smokers), more recent

occupational exposures at or below the current regulatory limits indicate that these represent levels that do not elevate cancer risk even for lifetime occupational exposures. Moreover, the previous exposures of concern in workers from the 1930's through the 1960's were at levels that typically exceeded 1,000,000 ng/m^3 and also involved exposure to the most carcinogenic forms of chromates, i.e., the insoluble or slightly soluble forms such as lead chromate, zinc chromate and calcium chromate. The newer lifetime occupational exposure limits -- at which no increase in cancer risk or other health effects has been observed – represent daily exposures that are hundreds to thousands of times higher than would occur in an environmental setting or via U.S. drinking water. There are two other major conclusions that can be drawn from these occupational exposure studies. First, although dermal exposure to chromium(VI) was extensive – particularly prior to the advent of industrial hygiene practices in the 1970's – there is no evidence for increased risk of skin cancer, even in workers where the chromate levels were high enough to burn "chrome holes" in their skin or nasal septum. These chrome holes healed and were not associated with increased skin cancer risk in these workers. This is relevant to the very high doses of chromium(VI) used in the NTP studies and a possible MOA. Second, taken together these occupational studies do not demonstrate an increased risk of GI cancers or other internal cancers, despite the fact that these workers swallowed a significant fraction of the dusts they were exposed to in the air. These data were recently summarized in a meta-analysis published in 2010 (NM Gatto et al., Cancer Epi 2010, 34:388-399).

Other specific edits and comments (changes underlined):

- Page 200, Lines 1-2 – Delete the phrase *"... and evidence of an association between oral exposure to hexavalent chromium and stomach cancer in humans"* which is based on a single study and EPA's selective treatment of this result as discussed above.
- Page 200, Lines 18-20 – *"This study found evidence <u>of a modestly increased incidence of stomach cancer mortality (OR 1.69, CI 1.12-2.44)</u> from 1970 to 1978 ...."*
- Page 201, Lines 20-23 – *"... was <u>reported in a re-examination of a single study</u> in JinZhou .."*
- Page 209, last section, Bioavailability – This section is highly flawed in logic and presentation as discussed under Chapter 3. Specific edits are as follows:
    - Page, 209, last two lines – *"Quantitative studies of GI absorption <u>of chromium administered as hexavalent chromium</u> have estimated ..."*
    - Page 210, first line – Please note that hexavalent chromium was not measured, and without exception has never been measured systemically as <u>Cr(VI)</u> following GI absorption. It is an assumption that increased chromium uptake to the blood represents Cr(VI) uptake, but it could also represent other forms as discussed in Chapter 3 above. We know that certain forms of Cr(III) are much more readily taken up than others, and it is therefore possible, and perhaps quite likely, that reduction of Cr(VI) in the gut in the presence of organic molecules in the GI lumen leads to formation of complexes that are much more bioavailable than inorganic Cr(III) that is

typically found in food, water and soil. You could also modify this phrase as a separate sentence to read *"This may indicate that not all hexavalent chromium is reduced by the gastric juices of the stomach, or that reduction of Cr(VI) in this environment leads to formation of organic chromium complexes that are more readily absorbed than inorganic Cr(III)."*

- Page 210, Lines 7-12 - Delete the sentence beginning *"Thus, at oral doses within human exposure ranges ..."* as per the argument above or modify to include alternative interpretations as suggested above.
- Page 210, Lines 28-30 – End of sentence beginning with *"... and uptake of hexavalent chromium into the tissues ... "* delete this phrase or modify it as per the argument above.
- Page 210, last line – Add a sentence after the last sentence on this page reading *"However, none of those studies speciated the chromium that was absorbed systemically, and so the form(s) of chromium in the blood and other tissues is unknown following increased absorption of chromium following ingestion of hexavalent chromium. Therefore, it is not known whether chromium reaches the blood or distal tissues as chromium(VI) at doses relevant to human exposures. The lack of long-term labeling of RBCs by chromium in the animal and human studies argues that little, if any, chromium is absorbed as chromium(VI) under these exposure scenarios."*
- Page 213, Section 4.7.3.5. Lines 3-5 – This statement is completely incorrect; there is no evidence for it as argued above. Delete this, or modify as follows *"Chromium absorbed following ingestion exposure to chromium(VI) may be in forms that can reach the systemic circulation and distal tissues, thereby potentially affecting tissues beyond those at or near the site of entry. However, the form(s) of chromium following such uptake is not known."*

Chapter 5

This reviewer strongly objects to use of a linear low-dose approach for Cr(VI) risk assessment given the clear evidence for a threshold mechanism due to extracellular reduction of chromium at doses of relevance to human exposure via drinking water. The conclusion by EPA of a mutagenic action of chromium – most of which is based on cell culture data where chromium exposures and other parameters were extreme and where metabolism and intracellular exposure are far different than in vivo exposures – should not be the sole basis for use of this standard model which ignores the compelling toxicokinetic data summarized in Chapter 3 of this draft. More importantly, as discussed above for Chapter 4, Cr(VI) is unlikely to act via a mutagenic MOA in vivo, and requires extraordinary experimental manipulation to be positive in cell culture and in vivo mutagenicity studies. While it is clear that Cr(VI) can cause certain forms of DNA and chromosomal damage or other changes, it is not clear whether any of these is pre-mutagenic, and the in vivo data argue strongly against a mutagenic MOA under physiological conditions and normal routes of exposure. The document must more clearly differentiate between genotoxicity – i.e., damage to DNA or chromatin – and mutagenicity

– or frank mutations that may result from DNA damage. Chromium(VI) can induce DNA damage but is a very weak mutagen at best, particularly in vivo. It is far more likely, and most consistent with all available data, that chromium(VI) acts via a non-mutagenic mechanism that involves a clear threshold – two threshold actually, one of which is extracellular and chemical involving reduction of chromium(VI) to chromium(III) and the other of which is biochemical and involves a threshold for cellular effects that lead to cell damage and cell death, resulting in turn in tissue proliferative responses that ultimately increase tumor risk via well known mechanisms of repeated tissue injury, compensatory cell proliferation and re-population.

Given this most likely MOA based on a synthesis of several decades of chromium research, it is therefore inappropriate to use a linear low-dose extrapolation model for assessing risk via the ingestion route of exposure. It is clear from the animal and human studies that there is a threshold for in vivo effects that is based on the strong reducing capacity of the GI tract following oral exposures, and that at normal drinking water concentrations this will effectively protect from any in vivo exposure to Cr(VI) as Cr(VI). Thus, a more appropriate risk assessment method would be to do dose-response modeling from the 2008 NTP study and the more recent ACC-sponsored 90-day MOA studies, and then use an approach similar to that for the RfD to calculate, with appropriate safety factors, a drinking water MCL that is protective based on threshold mechanisms. This should be done for Cr(VI) rather than the current MCL that is for total chromium, but it is likely that an MCL in the range of 50-100 ppb is going to be fully protective, including several uncertainty factors that separate it from the departure point of any likely human health effects for even the most sensitive individuals.

Chromium is an excellent example of an opportunity to apply the concept of evidence-based risk assessment – which the EPA has claimed to be promoting for many years but has not, to date, actually applied in any meaningful risk assessment -- since there is a strong and compelling argument for use of a non-linear, threshold-based mechanism for chromium that logically leads to a real-world risk assessment that is based on that mechanism. Setting aside the 1987 Zhang and Li study and subsequent re-analyses as per the arguments above, there is not a single credible epidemiology study linking exposure to chromium via ingestion with cancer risk or any other long-term health effects, including in the extensive occupational epidemiology literature which includes several decades of extremely high-dose exposure cohorts. And even taking into account the Zhang and Li study, there is not a single peer-reviewed report linking any health effects to chromium(VI) at levels within a hundred-fold of likely environmental exposures via drinking water. The NTP 2008 animal data showed evidence of a cancer increase only at the highest doses, and the more recent ACC studies demonstrated hyperplasia consistent with a non-mutagenic MOA, which is also consistent with a threshold mechanism and which argues against developing a linear cancer slope factor from those data.

Chapter 6

There is considerable disconnect between the conclusions provided in Chapter 6 and the more considered and detailed discussions of the primary data in the previous chapters. The language should be modified to reflect this understanding such that the conclusions and their application to risk assessment of chromium(VI) follow logically from the scientific evidence. Specific edits:

- Page 235, Lines 18-19 – " *... resulting in substantial, and in some cases complete reduction of hexavalent chromium to trivalent chromium depending on the concentration, dose, and precise route and method of exposure."*
- Page 235, Lines 21-26 – *"The extent of absorption of chromium from ingesting hexavalent chromium appears to be determined by both the solubility..... in the GI tract, but ingestion of both trivalent and hexavalent chromium results in systemic uptake of chromium. Trivalent chromium does not readily cross cell membranes except as part of certain organic complexes. Hexavalent chromium, if absorbed systemically, can easily ...."*
- Page 235, Lines 27-29 – *"Chromium absorbed systemically from the gut following ingestion of hexavalent chromium is distributed throughout the body. .... If hexavalent chromium is absorbed without extracellular reduction, it can cross cell membranes and, once inside the cell, ..."*
- Page 236, Line 3 – *"Chromium absorbed systemically following hexavalent chromium ingestion is eliminated primarily in the urine as trivalent chromium."*
- Page 239, Lines 11-12 – delete the phrase *".. and evidence of an association between oral exposure to hexavalent chromium and stomach cancer in humans."* This is a significant statement to make, and as discussed above in reference to Chapter 4, the Zhang and Li studies (1987, 1997) should not be used to assess human cancer risk for oral exposure to chromium.
- Page 240, Lines 12-14 – As noted for Chapter 5 above, there is considerable concern with the default choice of a linear low-dose extrapolation model for cancer risk.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Hexavalent Chromium

A1. A two-year drinking water study of sodium dichromate dihydrate in rats and mice (NTP, 2008) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

A2. Diffuse epithelial hyperplasia in the duodenum of female mice was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Joshua W. Hamilton, Ph.D.

A3. Benchmark dose (BMD) modeling was applied to the incidence of diffuse epithelial hyperplasia in the duodenum of female mice to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of diffuse epithelial hyperplasia) scientifically supported and clearly described?

A4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

Reviewer Response:

No concerns regarding A1. And A2. This reviewer would point out that the hyperplasia that was chosen as this endpoint, while appropriate for this RfD, is also appropriate for considering the carcinogenic MOA as well, as argued above and below and taking into considering the recently reported ACC studies that should be considered in this regard. This reviewer is not an expert on modeling and cannot comment on A3 in detail. No concerns regarding A4. Uncertainty factors are policy decisions, not scientific ones, and we can neither prove nor disprove any of the assumptions on which they are based nor can we accurately determine when and how such factors might be applied. Based on previous EPA doctrine, these seem to be consistent with previous applications.

(B) Carcinogenicity of Hexavalent Chromium

B1. Under EPA's 2005*Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgrd.html), hexavalent chromium is *likely to be carcinogenic to humans* by the oral route of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described?

B2. A mutagenic mode of carcinogenic action by all routes of exposure is proposed as the primary mode of action for hexavalent chromium. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for hexavalent chromium that may support an alternative primary mode of action.

B3. A two-year drinking water study in rats and mice (NTP, 2008) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantification is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

B4. The incidence of adenomas and carcinomas combined in the small intestine of male mice from the NTP (2008) two-year drinking water study were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

B5. The oral slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk of tumors of the small intestine in male mice). Has the modeling been appropriately conducted and clearly described?

Joshua W. Hamilton, Ph.D.

Reviewer Response:

Regarding B1. and as outlined in the detailed comments under G1. and G2., this reviewer is concerned that the evidence for carcinogenicity is not strong in animals and is not supported by human epidemiology. As also noted in comments under G1. and G2., there is considerable concern with statements by EPA in sections of this draft, particularly under Major Conclusions in Chapter 6, regarding evidence for human carcinogenesis based on a single human epidemiology study that in turn is a re-analysis of another study that lacks critical information that would be useful in fully assessing relative cancer risk. However, based on the current criteria for selection of this designation, it appears to be consistent with EPA doctrine since there is evidence of increased tumors in animals under certain exposure conditions.

B2. There is considerable evidence that Cr(VI) is genotoxic in cell culture and in vitro, and under certain extreme conditions it can also be shown to be mutagenic. However, there is far less support that Cr(VI) is genotoxic or mutagenic in vivo by the oral route of exposure at doses of relevance to humans; conversely, there is considerable evidence that there are protective threshold mechanisms that significantly impact the ability of Cr(VI) to reach target tissues and cause DNA damage under physiological conditions. In addition, while alternative mechanisms are briefly discussed, these are essentially dismissed without extensive treatment. As noted in the document, there is not a large literature on alternative mechanisms, but this is largely because, since the discovery of the genotoxic potential of Cr(VI) some forty years ago, most of the field has only focused on this one aspect, and I suspect it would be very difficult to get peer-reviewed funding to study non-genotoxic mechanisms for this toxicant. It is important to note, however, that there are no reports of increased skin cancer under occupational exposure settings, despite the fact that workers until the past few decades were directly exposed to Cr(VI) on the skin to the extent that they formed "chrome holes" that eventually healed. Yet this direct application and clear signs of chromium reduction and toxicity directly on the skin produced no increased skin cancer risk. Likewise, the occupational exposure literature only recently investigated the role of smoking status in chromium-related lung cancer risk. The increased risk of lung cancer associated with Cr(VI) exposure is modest considering the exposure levels and duration of exposures that span decades, and virtually all of the cancer cases in the epidemiology studies were seen in smokers, suggesting an interaction but one that is very modest. This in turn suggests alternative mechanisms such as inflammation, oxidative damage, damage-induced proliferation, and other mechanisms not directly tied to the ability of Cr(VI) to enter cells and damage DNA. These should considered and explored in more detail, since they are the basis for many of the assumptions regarding the risk of cancer from oral exposure to Cr(VI).

B3. There are no significant concerns about selection of this study, it is clearly the best available study for this type of risk assessment, with the caveats about how these data are interpreted and modeled as discussed elsewhere.

B4. There is concern about selection of these endpoints to represent cancer risk in these animals as the basis for a human risk assessment. The high doses required to induce these lesions are well above a threshold level that would be of concern in humans under normal exposure scenarios, and these almost certainly represent a scenario where natural reductive defense mechanisms were overwhelmed by the doses of chromium used. Taken together with the more recent 90-day ACC sponsored studies, the MOA for chromium(VI) is most likely a non-mutagenic one involving tissue damage and reproliferation, and would only be seen at doses that are unlikely to ever occur in a human exposure setting, particularly via drinking water. Thus, a threshold based risk assessment is most appropriate, similar to the treatment used for the non-cancer endpoints which are likely to be directly related to the cancer MOA.

B5. As discussed in detail under G1. and G2. responses, this reviewer has considerable concerns about the use of a linear low-dose extrapolation model for assessing chromium cancer risk. The evidence, when objectively assessed, strongly argues for threshold mechanisms both in the gut and systemically, and there is little or no evidence that chromium reaches the systemic circulation as chromium(VI) under exposure scenarios of relevance to human exposures. There is no better candidate for departure from these default EPA assumptions than chromium if EPA is serious about evidence-based risk assessment. Many of the public comments that were available to the reviewers just before and after our May 2011 meeting also raise these issues, and the EPA should, in particular, wait until the recently reported 90-day MOA and PK studies are published and available to them, at which point they should give serious consideration to how these new data inform the likely MOA. Given that most toxicology profiles are only revised every 10-15 years, it is worth waiting for these studies, and taking them as well as the external reviewer comments in mind toward a revised document that will be more accurate and will better stand the test of time. The EPA might also consider asking for a National Research Council Special Emphasis Panel to review all these materials and make a recommendation to EPA regarding chromium(VI) as has been done for other several other key toxicants of concern. In any event the current draft's risk assessment treatment of chromium(VI) is highly flawed and grossly mischaracterizes the likely risk of human health effects of chromium(VI) in drinking water based on a careful and thorough assessment of all the available evidence.

POST-MEETING COMMENTS SUBMITTED BY

Monica Nordberg, Ph.D.

Monica Nordberg, Ph.D.

Charge Questions

General Charge Questions:

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazard?

EPA has presented and synthesized present knowledge about non-cancer and cancer hazards for hexavalent chromium. However, some further literature could be included in the document and paid attention to i.e, Langård and Costa Chapter 24 Chromium In: Nordberg GF, Fowler BA, Nordberg M and Friberg LT (Eds.) (2007) Handbook on the Toxicology of Metals, 3rd edition, Elsevier 487-510.

Other chapters of interest e.g.,Chapter 10 Carcinogenicity by Ke, Costa and Kazantzis page 177-196. One chapter (14 by G. Nordberg and B A Fowler) deals with Risk assessment pages 281-301.

A comparison between criteria for classification of carcinogenicity should be done between IARC, EU and USA. Hexavalent chromium is classified as a human carcinogen. This evaluation is also taken by USEPA for inhaled hexavalent chromium and related lung cancer. It should be highlighted that for some metals e.g., arsenic it has been reported in the scientific literature that oral intake i.e., via drinking water also can give rise to lung cancer though oral intake mostly is referred to cancer in the oral cavity or gastrointestinal system.

My question is if the lung was studied in the NTP studies or any of the animal studies reported in the given report? Same question goes for the epidemiological studies that are cited?

Table 3-7 page 30 reports levels of chromium in female controls both in kidney and in bone. It is not easy to find any comments on this in the document. Is there any analytical problem in this study?

G2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

To my understanding there are new experimental studies with more for this purpose fitted doses. It could be worthwhile to await the outcome of these studies to find out whether more appropriate values for both NOAEL and LOAEL will be reported and include these in an appendix if feasible.

Monica Nordberg, Ph.D.

Any further data obtained in the cited NTP studies should be included and presented to the reader. It would be of interest to know if there are any data on lung cancer or other effects from the NTP studies.

During the workshop a number of ongoing studies were presented and it was suggested that they be paid attention to. It is always an advantage to get more and more information and research is always going on.

In my opinion it is however important to set recommendations for exposure to toxic agents in order to protect humans from developing adverse health effects. It is a human right to be protected from unwanted exposure which also will cause unnecessary worry during the time from alert to protection. People expect regulatory agencies to make evaluations and set exposure limits. Studies underway even if published in peer review scientific journals should be carefully evaluated and scrutinized by EPS´s working group to determine if presented data is reliable e.g., based on a number of factors such as, just to mention a few, how large are the studies and what is the power o the study, analytical procedures that include quality control so data is validated and to be trusted. Based on experience it takes time before data will be available even for ongoing studies. I recommend that IRIS, EPA sets a recommendation based on information presented in the draft document. In case important information which can change any evaluation shows up in time, such data can be included in the final document as an appendix or addendum. It is important in Risk Assessment to keep in mind that any recommendation set for exposure levels values needs to be reevaluated over time because by new techniques e.g., rapid development of usage of "omics" has to be considered. In view of said it is important to draw conclusions now and on data available now and not to wait.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Hexavalent Chromium

A1. A two-year drinking water study of sodium dichromate dihydrate in rats and mice (NTP, 2008) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

EPA suggests an oral RfD of $9x^{10-4}$ mg/kg-day.

The epidemiology studies in Liaoning province, China (p 68-76 in draft report) reported increased incidence of cancer after intake of drinking water contaminated with hexavalent chromium. It is stated in the document to be the only reported human data. That study supports the statement of hexavalent chromium in drinking water to be carcinogenic.

It should be explained to the reader why sodium dichromate dehydrate was chosen for oral exposure study. Data in the literature indicates that bioavailability and bioaccesibility depends on species of the compound and also exposure media. Are there any data on hexavalent chromium species in the drinking water in the general environment? The NTP studies that are reported have used doses that are much higher than reported present concentration in drinking water in the general environment. Thus values for LOAEL is identical to lowest administered dose. It has not been possible because of applied doses to set a NOAEL.

A2. Diffuse epithelial hyperplasia in the duodenum of female mice was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Perhaps some information on possible effects on the lung should be comment on. The document should give information about solubility of different chromium (VI) species should be given and specifically for the chromium species that have been used in the quoted studies. Soluble salts are mentioned on page 54 under 7 but soluble in what media is not mentioned. The reference WHO/IPCS (2006) Environmental Health Criteria 234, Elemental speciation in Human Health Risk Assessment, WHO, Geneva is recommended to be included.

A3. Benchmark dose (BMD) modeling was applied to the incidence of diffuse epithelial hyperplasia in the duodenum of female mice to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of diffuse epithelial hyperplasia) scientifically supported and clearly described?

Yes

A4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

The reason for chosen UFs is clearly and properly described.

It is known that the reduction of hexavalent chromium to chromium three is influenced by vitamin C. This can perhaps be used in setting UFs and thus not only choose the standard UFs of 10 between species and 10 for interindividual differences. Humans can not synthesize vitamin C and are thus depending on vitamin C supplementation. The tested animals i.e., the mouse and the rat both produce vitamin C themselves. In this context a laboratory animal that resembles the human by being depended on vitamin C supplementation might be used in future studies. The concentration of vitamin C in tissues and organs are very important in

evaluation of carcinogenic metals. It is likely to be involved in the mechanism in causing cancer and plays a role in the MOA.

(B) Carcinogenicity of Hexavalent Chromium

B1. Under EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgrd.html), hexavalent chromium is *likely to be carcinogenic to humans* by the oral route of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described?

Though it is an American document prepared for US I would recommend also to consult and cite documents published by the United Nations Organizations such as International Agency for Research on Cancer (IARC) and World Health Organization (WHO). Recommended literature is IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 49 (1990), Lyon, France and Chromium, Nickel and Welding 1-677 pages and IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 100 which is in preparation and further information on www.iarc.fr

Hexavalent chromium is classified as a human carcinogen. It is not clear to the reader why classification can be different upon different route of exposure.

B2. A mutagenic mode of carcinogenic action by all routes of exposure is proposed as the primary mode of action for hexavalent chromium. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for hexavalent chromium that may support an alternative primary mode of action.

Again this might be linked to differences among hexavalent chromium species regarding for example solubility in body fluids.

Somewhere in the document it should be pointed that iron can reduce hexavalent chromium to chromium three. This has been done in some products. This is touched by on page 48 line 18.

The document describes in detail the possible MOA. IARC 1990 stated "... chromium (VI) compounds on the basis of the combined results of epidemiological studies, carcinogenicity studies in experimental animals, and several types of other relevant data which support the underlying concept that chromium(VI) ions generated at critical sites in the target cells are responsible for the carcinogenic action observed"
However, also alternatives like DNA- methylation and other epigenetic mechanisms should be considered, because for many metals DNA-methylation is recognized as a possible mode of action also addressed as mechanism for carcinogenicity. Effects on cell signalling and gene expression may also serve as mechanisms

involved in carcinogenesis of metallic compounds. See further discussion in review by Davidsson et al, Chapter 5 in Nordberg et al (eds) Handbook on the Toxicology of Metals, p79-100

B3. A two-year drinking water study in rats and mice (NTP, 2008) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantification is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

In this document it is not explained to the reader what decided the selection of chromium species and selection of doses and how the chosen doses relate to exposure in drinking water in the general population. Lethal doses should also be given for the chromium species that have been used in the quoted studies. To evaluate and compare the outcome of studies and concentration levels in tissues, a ratio of concentration in dry weight to concentration in wet weight would make it possible and easier to compare reported results and also of possible intake of hexavalent chromium in drinking water. It is told that the animals by increasing exposure to hexavalent chromium in drinking water showed a decrease in intake of drinking water. Influence on different tissues will be found in doing this. On page 112 NTP 2008 decreased body weights could be explained by reduced drinking water consumption.

B4. The incidence of adenomas and carcinomas combined in the small intestine of male mice from the NTP (2008) two-year drinking water study were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

See comments above. It should be noted that pH is different in different parts of the gastrointestinal system.

B5. The oral slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk of tumors of the small intestine in male mice). Has the modeling been appropriately conducted and clearly described?

The model is clearly described. However I feel very uneasy about extrapolation to lower exposure levels because of chosen exposure doses where LOAEL is identical to the lowest exposure dose administered. Other organizations like IARC do not perform any quantitative evaluation of carcinogenic agents/substances. Threshold concentrations are problematic because of lack of knowledge of how the carcinogenicity develops. Once an organism has been exposed to a substance that can give rise to cancer there is a possibility to such an effect to occur.

POST-MEETING COMMENTS SUBMITTED BY

Steven R. Patierno, Ph.D.

Steven R. Patierno, Ph.D.

Toxicological Review of Chromium

Charge Questions

General Charge Questions

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for non-cancer and cancer hazard?

I will offer a response to this question in the form of general comments regarding specific sections of the Review in order of appearance in the text. In taking this approach my comments will also directly address questions (A)1-4 and (B)1-5.

Page 7: The Environmental Protection Agency (EPA) should not be referencing a 2006 review article by Costa and Klein to site background on environmental chemistry. This review article was not a critical review of the environmental chemistry of chromium. Even if the general background in that review article is accurate, The Toxicological Review of Hexavalent Chromium (TRHC) should cite primary references from chemical or environmental journals or compendiums. Also, this paper is mis-labeled in the reference list as a 2008 paper. [Correct reference is "Toxicity and Carcinogenicity of Chromium Compounds in Humans" Crit. Rev. Tox.: 36(2):155-163, 2006].

Moreover, the premise of this review article [in essence that even very low exposures to any form of CrVI, including in drinking water, can cause virtually any type of cancer in virtually every organ, as well as plethora of assorted other diseases], and the preceding review article on which it was largely based ["Toxicity and Carcinogenicity of Cr(VI) in Animal Models and Humans" Crit. Rev. Tox.: 27:431-442, 1997], should not be universally accepted by the EPA without critical evaluation. Much of the epidemiological methodology applied in these papers is flawed. In these papers, the author(s) repeatedly and selectively tabulated whatever instances could be found in any of the many epidemiologic studies of chromium, of an elevated Standard Mortality Ratio (SMR). These were presented with no mention of the fact that most of these instances were small, non-statistically significant elevations (likely to be random fluctuations due to the large breadth of the studies), which were either ignored or discounted by the original authors because of confounding factors. The paper also failed to take into account that many of the small, non-statistically significant elevations in some cancers in one selected study, were counter-balanced by either no elevation or decreased SMRs in other studies. This "tabulation" approach does not constitute a true meta-analysis and is also statistically incorrect.

There are also additional reasons that the EPA should be circumspect about citing either of these articles. The 2006 article, and its preceding counterpart published in 1997, were written and published at a time when the senior author was actively engaged as an expert witness for the plaintiffs in high-profile hexavalent chromium lawsuits. This involvement was not disclosed in the 1997 article, which was focused on attempting to implicate low dose exposure to CrVI in a broad array of human cancers. In the Acknowledgements section of the 2006 article there is partial disclosure that production of the paper was paid for in part by Baron and Budd. In fact, Baron and Budd is one the law firms with whom the senior author was under contract with as an expert witness for the plaintiffs in an active lawsuit. This article specifically tried to implicate CrVI as a human drinking water carcinogen even at very low doses, as well as suggesting that exposure to CrVI causes a broad array of other diseases, including neuropsychiatric problems, for which there is no support. If the EPA is going to site these review articles it is critical that EPA conduct an independent critical review of every paper sited in these review articles. In the latter scenario it is certain that EPA will reach a different conclusion.

I am not of the opinion that a scientist who serves as an expert witness should have to disclose all litigation-related work in all scientific publications, particularly not in reports of original laboratory research into mechanisms of action, or even in review articles that give an unbiased evaluation of the existing literature, especially as it relates to basic mechanisms of action. Indeed, in the world of chromium toxicology it is hard to find experts who have not participated in some sort of chromium-related litigation. However, these two articles do not merely describe original laboratory research or present an unbiased review of the literature (note that part of the 2006 review article is a recapitulation of an already published journal article on UV light and chromium exposure). These two particular articles are essentially position/opinion papers, with speculative declarations that even very low dose exposures to soluble CrVI can cause virtually any kind of cancer (and other disease) in virtually any organ, a theory of obvious benefit to any plaintiff's case in chromium-related litigation, but one that is not supported by either epidemiological studies or in vivo animal studies.

Page 20-21: Although the draft TRHC frequently describes each specific study in this section and offers a conclusion/interpretation, the TRHC discussion of the Donaldson and Barrera paper ends with a reference to Table 3-1 with no summary. There is important information in Table 3-1 that strongly supports the capacity of gastric juice to rapidly reduce CrVI to CrIII. Note that the uptake of Cr in intestinal rings was virtually identical, whether the starting material was CrIII without gastric juice or CrVI plus gastric juice at pH 1.4.

The text at the bottom of page 21 and the top of page 24 seems to be nuanced to cast doubt on the body of work of DeFlora, by using the word "suggested" (second line from the bottom of pg 21), and then suggesting

that the values of reducing capacity given by DeFlora "should be considered with some caution". This "caution" is based on speculation found in the paper cited (Zhitkovich, 2005), and reiterated in the 2006 Costa and Klein article (from where the draft TRHC apparently drew its language). This speculation is addressed in the supplementary materials under "Public Comments". The TRHC and the EPA should not cast doubt on the body of work by DeFlora, based on unsubstantiated speculation.

Page 24-5: The TRHC should recognize and illustrate the main point of the absorption studies cited: no matter whether the original starting material is CrVI or CrIII there is limited absorption and little retention of either: fecal recovery in rats was 98% for CrIII and 97.7% for CrVI (pg 24) and in humans was 99.6% for CrIII and 89.4% for CrVI. Pretreatment of CrVI with gastric juice completely inhibited absorption of CrVI after direct perfusion into the small intestine. On pg 27 another study indicates that 99% of CrVI is recovered in feces using rats gavaged with CrVI. On pg 28 another study indicates that maximal uptake after gavage of rats with CrVI occurred in liver and was only 1%. Absorption in other organs was in the range of 0.1 to 0.2%. It is important to note that in all of these absorption studies, including drinking water studies, the increased tissue distribution was only observed after chronic administration of more than 5 ppm. Many of the studies used greater than 100ppm. The main point that there is very little absorption and retention of Cr even after administration of CrVI.

Page 26: It is incorrect to state that absorbed "hexavalent" chromium is distributed throughout the body. Few studies actually speciated the Cr found in organs distal to the route of administration and even extremely large doses of CrVI, large enough to saturate reduction in the stomach and GI tract, do not deliver much more than trace amounts of CrVI to most distal organs because of the vast reducing capacity of blood components. The vast majority of Cr reaching distal organs arrives as CrIII. The TRHC should make this absolutely clear.

Page 34: The TRHC should provide an accurate summation of Table 3-8 which compares tissue chromium after ingestion of a very large, gastric reduction-saturating dose (12.9 mg/L) of either CrIII or CrVI. The only "organ" that showed consequential increased levels of Cr after CrVI compared to CrIII was blood. Most of the other organs exhibited only trace amounts of Cr, even after this huge dose of CrVI, except for the intestine which showed significant and nearly identical increased Cr concentrations after both CrIII and CrVI. This supports the conclusions of the supplementary data in the Public Comments showing that some sections of the intestine (jejunum for example) are sites of Cr accumulation, regardless of whether the source material is CrIII or CrVI.

Page 35: Note that there is no increased accumulation after 8 weeks of exposure compared to 4 weeks of exposure, even at the enormous dose of 130 ppm (mg/L).

Steven R. Patierno, Ph.D.

Page 36: The NTRC should not interject the commentary statement: "indicating that a portion of the CrVI escaped intracellular reduction in the GI tract and became bioavailable for systemic distribution". Like almost every other study this study measured total tissue Cr and did not speciate tissue Cr, and the NTRC therefore cannot speculate on what the form of Cr was that reached the tissues. Note in Table 3-8 the accumulation of Cr in the intestine and blood after CrIII.

Page 37: Note the obvious threshold of increased bone and kidney concentrations after 10 ppm compared to all lower doses, as well as estimated body Cr burdens. Note that in liver there is a significant Cr burden even after ZERO CrVI exposure. Note the strangely compressed scale of the Y axis: even the increase in females at 10 ppm is only an increase from 0.3 to less than 0.5. These data demonstrate the exact opposite of the conjecture on page 36: there is a clear threshold of accumulation indicating saturation of reductive capacity.

Pages 38-39: Virtually every study shows the same thing. The NTP study used a "low dose" that is already higher than the 10 ppm in the Sutherland study. What is being referred to here as a "dose-dependent" increase is already supra-saturation of gastric reductive capacity. What these studies really show is how little Cr is absorbed, even in tissues that are directly exposed (glandular stomach and forestomach), even after massive doses are administered.

Page 41: It is inappropriate to make such an unqualified statement as found at the top of this page: "Hexavalent chromium is capable of crossing the placenta". This is only true in the highly contrived circumstances referenced below the statement wherein pregnant mice were given an IV injection of a massive dose of CrVI (10mg/kg).

Page 42: The TRHC does a good job describing the bioavailability and kinetics of Cr absorption in humans after CrIII, CrVI in OJ and CrVI. It correctly acknowledges that the CrVI-OJ was completely reduced and that even the full dose of CrVI was insufficient to overwhelm the reducing capacity of blood. The potential explanations offered are correct but need to add another possibility. Often overlooked is the fact that not all CrIII is alike. Anyone who works with CrIII in the laboratory knows that it undergoes aging in aqueous solution, even visibly changing color with time after solubilization. It is possible that CrIII generated from newly reduced CrVI (as in the CrVI-OJ) may have some different biological parameters than straight CrIII made up in water and allowed sit for a couple of days. In fact overall absorption of newly formed CrIII may be higher than aged CrIII, possibly as a function of its ability to form complexes with biological ligands that may alter its absorption potential.

Page 49 bottom: It is inaccurate to state that "CrVI distributes to other tissues, notably the blood, kidney, and liver." Except for the cases of treatment with extreme doses, or use of pathways like intra-intestinal instillation or IP injection, the vast majority of Cr that arrives at distal tissues is CrIII. Once again, it is critical that TRHC make that fact clear, otherwise it gives the appearance of non-objectivity.

Page 50, last paragraph: The TRHC should not simply reiterate speculation that is found in the papers it cited (Zhitkovich 2005, Costa and Klein 2006), in suggesting that the mutagenicity of Cr may be underestimated in cultured cells because of lower levels of intracellular ascorbate when cells are cultured in absence of added ascorbate. Indeed, it is just as likely that the mutagenicity of CrVI in cultured cells is grossly overestimated, because the lack of ascorbate in the extracellular medium allows CrVI to persist in the extracellular medium thereby maximizing its uptake as the hexavalent oxyanion. At the very least the TRHC should discuss both possibilities and not give the appearance of bias.

Page 58: It is inaccurate to state that model simulations "imply" that some CrVI escaped reduction in the stomach. This is circular reasoning. The "input" data that went into formulating these models was based on experiments wherein massive doses of CrVI were administered, doses that would clearly exceed reductive capacity. It is not appropriate to then state that the model simulation "implies" that some CrVI escaped reduction, as though the model now supports a novel biological observation. It would be completely expected for the model to predict that scenario since it would logically emanate from the very data that was used to formulate the model. It is critical that the TRHC indicate that these models do not apply to, or accurately predict, the toxicokinetics of low, environmentally relevant doses of CrVI. The discussion in the THRC does become more balanced on page 64 where the non-linear aspects of CrVI uptake, reduction and bio-distribution are given some weight.

Page 66: This section (3.6) needs to be completely rewritten as it lends undue weight to an opinion expressed in only one or two papers, at least one of which was written under financial inducement by a law firm with a vested interest in characterizing all Cr, including CrIII, as a potential hazard (see preceding comments). The TRHC needs to not indiscriminately cite speculation found in review articles without more rigorous analysis. Except for those few biased citations it is almost universally accepted that CrIII is an essential element.

Page 68: Section 4.1.2, last sentence: This is nearly the ultimate example of how critically important it is for the TRHC to do its own critical analysis of the literature. The paper cited, (Bick et al, 1996) should not be cited under any circumstances and in fact it should be retracted from the scientific literature. Two of the authors, Walter Lack and Thomas Girardi, were two of the 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 (Costa, Bick and Teitlebaum) 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, the draft TRHC does not yet, but should reference the recent work of Dr. John Morgan, an epidemiologist for the California Cancer Registry. He has been tracking cancer incidence in the town of Hinckley CA (the "Brockovich" town) from 1996 to present. He recently published data showing that from 1996 to 2008, not only is there no excess of total cancer or any specific cancer in Hinckley, there are actually fewer cancers than expected.

Pages 71-80: The draft TRHC conducts a very thorough depiction of the different interpretations of the Liaoning Province situation. What seems to get lost in the details is the larger picture. This is a Province of a country wherein the background rates of both stomach and lung cancer are high even in non-chromium exposed comparison groups, indicating the presence of other contributing risk factors. This is a situation where exposure is characterized in terms of high dose, long term "yellow water", yet despite this potential significant exposure, the question of whether there is an additional modest increase in risk for stomach cancer hangs on whether a particular industrial area is included in the comparison group or not. There is much controversy surrounding the reports of cancer risk in this Province, but after discussing the controversy the draft THRC aligns itself with the method of re-analysis of Beamont et al. The THRC should then also cite the commentary by Allan Smith [Epidemiology 19:24, 2008] which accompanied the Beamont article: although Smith is sympathetic to Beamont's attempt to re-analyze the data, he also describes the extensive weaknesses of the approach. This is not the kind of data that a regulatory decision should be based on.

Page 81: The NTP toxicology studies on subchronic oral exposure (Section 4.2.1) are technically well done. The principle issue that needs to not be lost in the detail is that the lowest dose was 62.5 ppm, an enormous concentration of little or no environmental relevance. This is a "yellow water" situation to the extreme. 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. The extensive new data provided by ToxStrategies, described in the Public Comments, needs to be incorporated into the TRHC.

Page 84: Again, 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 (see below). 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 (pg 86).

Page 87-108: The results 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 emphasized here 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) (Tables 4-12, 4-13, 4-14). 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 should be that these are massive doses and they are eliciting minimal effects. This important concept should not be lost in the mass of detailed results.

Pages 109-120: 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 and Klein article, especially in criticizing the work of DeFlora. In point of fact, the results of the NTP assay, and the extensive additional data found in the Public Comments generated by a group of investigators around the country funded by ToxStrategies, 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 profieration, (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.

Pages 122-149: For these studies on the potential reproductive toxicities caused by CrVI one can only hope that the TRHC and EPA will remember the 16th century adage of Paracelcus "all substances are poisons, the right dose differentiates a poison from a remedy". These studies show reproductive toxicity at huge doses of CrVI, often given using invasive administration procedure (IP or IV injection), with little relevance to environmental exposure levels.

Pages 176-178: The in vivo studies showing DNA damage or mutagenicity in cells peripheral to the point of administration of CrVI were only positive when massive doses of CrVI were administered by gavage, direct instillation, or intravenous injection. Although some studies claim to find mutations in the absence of cytotoxicity these are highly contrived systems: for example eye spots in offspring of pregnant female mice given huge doses (62.5 ppm) of CrVI in drinking water. All studies of mutagenesis in cultured mammalian cells, including human cells, demonstrate that mutagenesis is only observed at doses that produce some degree of cytotoxicity and replication arrest. It should also be clarified in the TRHC that DNA damage and mutagenicity should not be equated: while mutagenicity may result from DNA damage, the relationship is not simple or linear and is further complicated by DNA repair. Also, it is unclear whether all forms of DNA or chromatin alterations (collectively termed DNA damage) are pre-mutagenic. For example, in silico studies

on DNA-protein crosslinks suggest that under certain circumstances CrIII can serve as binary crosslinking agent between small peptides and DNA. However, in in vitro studies in cultured cells and in in vivo studies, it is not clear what is actually being measured by assays for DNA-protein crosslinks. This phenomenon may in fact only indicate that chromatin isolated from certain cells exhibits a higher degree of condensation during isolation, rendering chromatin proteins more difficult to extract. What appear to be DNA-protein crosslinks can be actually be observed in cells treated with agents that do not participate in or catalyze formation of an actual binary crosslink.

Pages 202-214 (section 4.7.3.2): Many of the preceding comments directly address and provide major qualifications to the MOA discussed in this section, including the interpretation of reductive capacity found in the Stout et al report of the NTP assay.

It is clear that the EPA is 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-relevent 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 and at high doses that evoke major amounts of cell death. However, 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 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 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 dis-similar to the high-dose rodent assays wherein tumors were only observed at doses that produced chronic and fairly severe tissue damage. This harkens to what is sometimes viewed as a landmark study of lung cancer and occupational exposure to high doses of Cr(VI) by Gibb et al. Occupational exposure to in the chromate production

industry was categorized into 4 quartiles. The lowest two quartiles are huge levels of exposure by "environmental" standards, orders of magnitude beyond the even the highest known environmental exposures. The lowest quartile of exposure was essentially a No Effect Level (no elevated risk) and the slightly elevated risk ratio in the second quartile was not statistically significant. Interestingly, of the total of 120 lung cancer cases found in chromium-exposed workers, 116 were also smokers.

The EPA may be under certain historical regulatory 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 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 etiology of how that particular cancer develops. It will have no relation to any chemically-specific mutations caused by Cr(VI) because Cr(VI) is an exceedingly poor mutagen. Even at the low end of very high doses there is NO MOA because there is NO toxicity, no mutagenecity, 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. If ever there was a textbook case to be made for a "threshold carcinogen", it is Cr(VI).

General Charge Questions:

G2. The TRHC should absolutely consider the extensive new data being provided by ToxStrategies and presented in part in the supplementary section under Public Comments.

Chemical-Specific Charge Questions:

A1. The two-year drinking water study of sodium dichromate dyhydrate in rats and mice (NTP, 2008) is the most thorough and technically well-conducted study available. It is likely the best study available for selection. However, the interpretation of and conclusions drawn from that study need to be re-evaluated in light of the issues raised in my preceding comments and the additional data shown in the Pubic Comments and coming available from a multi-institutional study sponsored by ToxStrategies.

A2. The selection of Diffuse epithelial hyperplasia in the duodenum of female mice as the critical effect for the RfD should be re-evaluated in light of the issues raised in my preceding comments. It must be considered in the context of the non-linear, dose-related issues discussed above regarding saturation of reductive capacity and definitive threshold data for toxicity and carcinogenicity.

A3. See answer to A2 above.

A4. The Uncertainty Factors must be re-evaluated in the context of the non-linear dose-response data, the clear evidence of thresholds for toxicity and carcinogenicity and the fact that these high-dose, supra-saturation experiments cannot be extrapolated linearly to low or vanishingly small doses.

(B) Carcinogenicity of Hexavalent Chromium

B1. The cancer weight of evidence characterization is not scientifically supported. The conclusion that hexavalent chromium is "likely to be carcinogenic to humans by the oral route of exposure" is not scientifically supportable given the issues raised in my comments above. The non-linear dose data in both the NTP studies and the data preliminarily discussed in the Public Comments clearly demonstrate that the toxicities and carcinogenesis observed at these extremely high, obviously supra-saturating doses, cannot and should not be extrapolated to lower doses. See detailed comments above.

B2. The determination of a mutagenic mode of action by all routes of exposure should be re-evaluated before proposing it as the primary mode of action. Nearly all indices (NTP studies, inhalation studies, mammalian cell mutagenesis studies etc) indicate that carcinogenicity of CrVI is only observed under exposure conditions that evoke cellular toxicity, inflammatory tissue damage, and tissue regeneration. The DNA damage and presumed mutagenicity (actually epigenetic or stochastic selection of cells that survived toxicity) of CrVI is only observed at doses that also cause cell death and tissue damage. In vivo, these effects are only achieved at very large doses that clearly overwhelm the reductive capacity of the oral cavity, stomach and blood components resulting in a sharp threshold of carcinogenesis only at the two highest NTP doses. I have spent more than 25 years studying the molecular mechanisms of CrVI genotoxicity and mutagenesis and I have a deep appreciation for its capacity to interact with cells and alter DNA and DNA replication and transcription. However, just because CrVI is capable of causing DNA damage and what we thought was "mutagenicity" (see above) in carefully contrived experimental systems, does not mean that it does so under physiologically and environmentally relevant conditions. It is much more likely that the chronic tissue damage, with accompanying inflammation and subsequent proliferative regeneration, possibly in the presence of unrepaired DNA damage, all of which are only observed at the highest doses, is the principle mode of action.

B3. See previous comments above. The NTP study is the best study available but the interpretation of the data and conclusions drawn from it are incorrect. Important supplementary data is preliminarily discussed in the Public Comments.

Steven R. Patierno, Ph.D.

B4. See previous comments above. The NTP study is the best study available but the interpretation of the data and conclusions drawn from it are incorrect. Important supplementary data preliminarily discussed in the Public Comments.

B5. See previous comments above. The linear extrapolation from the POD is not appropriate. CrVI toxicity and carcinogenicity demonstrates distinct non-linearity and there is little or no relation between what is observed at the highest doses in the NTP study and any physiologically-appropriate or environmentally-relevant exposure.

POST-MEETING COMMENTS SUBMITTED BY

Toby G. Rossman, Ph.D.

Toby G. Rossman, Ph.D.

Toxicological Review of Hexavalent Chromate

General Charge Questions

G1. Is the Toxicological Review logical, clear and precise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazard?

In general, this was a clearly written document. However, my area of expertise is in the mutagenic and epigenetic mechanisms of action of carcinogens, and I found many more problems in those areas than in the rest of the document. I will discuss these in section B2.

Here I will mention some errors in the rest of the text.

p. 38, end of 1st paragraph reads, "Uptake in guinea pigs did not appear to generally differ from that of rodents". The guinea pig (*Cavia porcellus*) is a rodent.

p. 46, section 3.3, refers to transport of the hexavalent chromium oxyanion (for clarity, should this read chromate/dichromate?) by sulfate and phosphate transport systems (should be pleural). It is claimed that this allows accumulation in cells at higher concentrations than the extracellaular concentration. Neither the transport systems nor the evidence for higher intracellular accumulation are referenced. Actually, what allows higher accumulations is the fact that Cr(6) is reduced in the cell to Cr(3), which cannot get out, so what accumulates is Cr(3).

p. 201, 1st full paragraph: It is claimed that the key precursor events leading to chromium-induced mutagenicity have been identified in animals. This is not so. It is not even true for mammalian cells in culture. Some ideas have been derived from cell culture studies (but with Cr-damaged shuttle vectors). Almost nothing is known about mutagenicity in animals, and nothing at all is known about the genetic changes occurring in animal tumors or in the target tissues.

p. 202. Section 4.7.3. For reasons that will become clearer in my response to B2, this section is very flawed. I will mention just one point here: The confusion between "mutagenic" and "genotoxic" must be cleared up throughout this section (as well as throughout Section 4.5.). The thinking on this issue is very sloppy. A mutagenic mode of action is just that: it requires mutagenesis.

G2. Additional studies that would make a significant impact on the conclusions of the Toxicological Review.

Some of these will be presented in section B2, as they pertain to mode of action.

It is extremely important that the new information supported by American Chemical Council (performed by ToxStudies and others) should be considered before the final document is completed. They address a number of missing data sets. It is already clear that proliferative increases occur in the mouse duodenum at doses of Cr(6) lower than those that cause tumors. Also, there is evidence for cytotoxicity at these lower concentrations that may be driving the proloferative responses.

The fact that Cr is an essential element needs to be addressed. What are the implications for a threshold?

It is possible that dietary Cr(6) is significant and should be evaluated. All parts of grain contain Cr(6) and 10% of the Cr in bread is Cr(6) (Mishra et al., Food Chem. Toxicol. 33:393-397, 1995; Soares et al., J. Agric. Food Chem. 58:1366-1370).

River waters have a median Cr value [which is probably Cr(6)] of 10 ppb (range <1-30), and even rainwater has a range from 0.14-0.9 (ATSDR, Chromium, Draft for Public Comment, online).

A recent meta-analysis of cancers of the G.I. tract among those occupationally exposed to Cr(6), concludes that these workers are not at greater risk than the general population (Gatto et al., Cancer Epidemiol. 34:388-399, 2010). Inhalation exposure usually leads to G.I. exposure, suggesting a possible threshold if the ingested dose can be estimated.

(A) Oral Reference Dose (RfD) for Hexavalent Chromium

A1. A two-year drinking water study of sodium dichromate dihydrate in rats and mice (NTP, 2008) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

This does seem like the best and most complete study to use.

A2. Diffuse epithelial hyperplasia in the duodenum of female mice was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

This seems like an appropriate choice, but it's outside my area of expertise.

A3. Benchmark dose (BMD) modeling was applied to the incidence of diffuse epithelial hyperplasia in the duodenum of female mice to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of diffuse epithelial hyperplasia) scientifically supported and clearly described?

This does seem like the best and most complete study to use (but it's a bit outside my area of expertise).

A4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

This is outside my area of expertise.

(B) Carcinogenicity of Hexavalent Chromium

B1. Under EPA's 2005*Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgrd.html), hexavalent chromium is *likely to be carcinogenic to humans* by the oral route of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described?

Given the fact that there is not enough human data to firmly establish carcinogenicity, but there is animal data, "likely to be" is reasonable but "possibly carcinogenic at high dose" would be more accurate.

B2. A mutagenic mode of carcinogenic action by all routes of exposure is proposed as the primary mode of action for hexavalent chromium. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for hexavalent chromium that may support an alternative primary mode of action.

By definition, the "mode of action" (MOA) of a carcinogen is "a sequence of key events and processes, starting with interaction of the agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation" (USEPA, 2005). For mutagenesis to be a carcinogenic MOA, the agent must at the very least cause heritable mutations in mammalian cells. The mutations should be induced in a concentration range with low toxicity (preferably similar to concentrations seen in human exposures), and the mutations should be induced in the target tissues in animal experiments and in humans. Human and animal tumors should also show genetic alterations consistent with the types of mutations induced by the agents, and these should be early events.

The information about Cr(6) is lacking for much of these criteria. In fact, the human tumor data support an epigenetic mechanism more than a mutagenic one.

Genotoxic is not the same as mutagenic, and sections 4.5 and 4.73 must be completely rewritten, as they consistently confuse these terms. Standard genotoxicity assays were not designed to inform specific modes of tumor induction. With the exception of mutagenesis, these other assays (non-mutagenic assays) do not measure heritable events, but rather measure evidence of DNA damage or its repair. Non-mutagenic assays include chromosome aberrations, micronuclei, comet assays, DNA lesion measurements, and DNA repair assays. These assays are useful for hazard identification or as biomarkers of exposure. They provide only supportive evidence that mutagenesis might be a MOA. DNA damage *per se* does not inform us about eventual heritable change, which is the true issue. Most (but not all) mutagens cause heritable changes in DNA sequences by causing damage to DNA (pre-mutagenic lesions) that is converted to mutation after cell division.

Table 4-21 should be deleted, as results in bacteria are not relevant to tumorigenic MOA. A simple statement that Cr(6) is mutagenic in bacteria should suffice (referencing a review such as Klein CB. "Carcinogenicity and genotoxicity of chromium" In: Toxicology of Metals (Chang LW, ed). Boca Raton, FL:CRC Press, 1996, pp.205–219.

Table 4-22 represents positive results in a group of assays that measure both mutagenic and non-mutagenic endpoints (including a paper on epigenesis, Klein et al., 2002, which should be removed, as this is not a genotoxic event; neither is disruption of mitosis, which can have many causes). Table 4.22 has neither information on the concentrations inducing positive results, nor on the toxicity of those treatments.

This table is also deficient in the most important results in mammalian cells, i.e. mutagenesis. All of the studies reported are mouse lymphoma cell studies, yet later in the document (page 203), reference is made to mutations at the HGPRT locus in "Chinese Hamster ovary cells (V79 and AT3-2)" V79 is not a Chinese hamster ovary cell, it is a Chinese hamster lung fibroblast. In fact, one such study using V79 cells (Sugiyama et al., Mutat. Res. 260:19-23, 1991) shows a modest positive effect only in a narrow concentration range. A CHO line (AA8) showed a small increase (3.4 fold) at a dose giving 75% survival (Brooks et al., 2008). There is a fuller discussion of chromate mutagenicity (with references) in: Klein CB. "Carcinogenicity and genotoxicity of chromium" In: Toxicology of Metals (Chang LW, ed). Boca Raton, FL:CRC Press, 1996, pp.205–219. This article points out that chromium compounds are mutagenic in a narrow dose range,

possible because of persistent toxicity after treatment (e.g. residual toxicity was seen a week after treatment of V79-derived G12 cells).

Chromosome aberrations and DNA strand breaks can occur as a result of cytotoxicity. Dead cells do not become tumors. Unless assays for cytotoxicity are performed, it is not possible to know whether DNA damage occurs in cells that can replicate to form clones. Traditional cytogenetic assays rely on short-term cell survival to generate the mitotic figures necessary for analyses; the long-term viability of these treated cells cannot be determined. Thus, the relevance of this kind of data for carcinogenic MOA is questionable. To measure cytotoxicty, the gold standard is clonal survival, a method that is common in gene mutation assays, but not in other genotoxicity assays. Short-term survival assays, such as MTT, neutral red, and trypan blue, as well as measurements of mitotic index that are commonly used in cytogenetic assays, fail to detect early or delayed apoptotic events. Trypan blue detects only necrosis. MTT and neutral red assays can be delayed to allow time for apoptosis to develop, at which point the results approach clonal survival (Komissarova et al., Toxicol. Appl. Pharmacol. 202:99–107, 2005). As mentioned above, Cr(6) causes delayed residual toxicity (Klein et al. Environ. Health Perspect. 102 (suppl 3):63-67, 1994), and thus clonal survival or at least apoptosis at later times after exposure, are essential in establishing cytotoxicity levels. Normal human fibroblasts show ~80% loss of clonality after a 25h exposure to 2 μM sodium chromate [Vilcheck et al., Environ. Health Perspect. 110 (Sup5):773-777, 2002].

Micronuclei can result from DNA damage or from malsegregation of chromosomes. It has been recommended that this assay should be performed under conditions of high survival (an increase of >90% in number of viable cells) and that markers for apoptosis and necrosis be included [Kirsch-Volders, et al. (2003) Report from the in vitro micronucleus assay working group. Mutat. Res. 540:153-163]. In the case of Cr(6), at lower concentrations, most of the micronuclei are kinetecore-positive, meaning that they arise from malsegregation and not DNA strand breaks (Seoane and Delout, Mutat. Res. 490:99-106, 2001; Figgitt et al., Mutat. Res. 688:53-61, 2010). Those that are kinetecore-negative (arising from chromosome breaks) occurred only at the highest concentrations. Thus, Cr(6) induces aneuploidy rather than DNA damage at lower concentrations (Holmes et al., 2010; Figgitt et al., Mutat. Res. 688(1-2):53-61, 2010). Aneugenesis is caused by alterations in proteins, not DNA, and has thresholds.

It should also be noted that the mouse lymphoma assay (MLA), chromosome aberration assay (CA) and micronucleus assay (MN) give a large number of false positives, even compared with the Ames test. Chemicals that are non-carcinogenic after thorough testing in both male and female rats and mice are often positive in these assays (Kirkland et al., Mutation Research 584 (2005) 1–256).

The Comet assay detects single and double strand DNA breaks as well as alkali-labile sites. Nucleotide excision repair (NER) and base excision repair (BER) of adducts can create breaks as intermediates. Single strand breaks are quickly repaired and are not regarded as significant premutagenic lesions. During apoptosis, DNA fragmentation into segments of 180 base pairs occurs, whether or not the apoptosis was induced by a genotoxic event (Choucroun et al. 2001, Mutat. Res. 478:89-96; Henderson et al.,.1998, Mutagenesis 13:89-94.) Necrotic cells also display DNA damage (Fairbairn et al., 1996 Scanning 18:407-416.). In order to avoid false positive responses, Henderson et al. (1998) suggests that the concentration of test substance should produce >75% viability.

In summary, standard genotoxicity assays from hazard identification exercises cannot be used to establish a mutagenic MOA, because these assays do not measure heritable events and because the doses used in such assays are usually too high.

Other MOA's have not been adequately considered. These include, for example, selection for Cr-resistance, resistance to apoptosis, and aneuploidy. The evidence for a mutagenic MOI is weak. Mutations can result from DNA damage, but can also be a secondary effect of the loss of mismatch repair, aneuploidy, and other types of genomic instability (in other words, it is a later effect). With the exception of the mouse lymphoma system, Cr(6) is only weakly mutagenic in mammalian cells, rarely giving more than a 3-fold increase in mutant fraction over background levels (in endogenous genes), and in a very narrow (and toxic) dose-range with a strong threshold (reviewed in Nickens et al., 2010).

In some cases the "mutations" have been shown to be epimutations resulting from altered DNA methylation (Klein et al., 2002). Since none of the other studies on mammalian cells looked for epigenetic inactivation, this calls into question whether the "mutants" seen are really mutants. These are important considerations for the MOA of Cr(6), since cells grown in the presence of Cr(6) show selection for cells with inactivated mismatch repair (MMR) genes. These cells are Cr(6)-resistant and could be the result of either mutation or epigenetic inactivation (reviewed in Salnikow and Zhitkovich, 2008). Cells with epigenetically inactivated MLH1 (a MMR gene) were seen in human lung A549 cells exposed to Cr(6) (Sun et al., Toxicol. Appl. Pharmacol. 237:258-266, 2009). MMR-deficient cells are mutators (having a high spontaneous mutation rate) and show microsatellite instability. An important consideration for MOA is the fact that chromium-induced lung cancer cells also show epigenetically-inactivated MMR genes (Takahashi et al., 2005). Also against the idea of a mutagenic MOI is the fact, discussed in Salnikow and Zhitkovich (2008), that Cr-induced lung tumors in humans lack p53 mutations, in contrast to lung tumors associated with other agents such as tobacco smoke, and the fact that the few mutations found do not correspond to the types of mutations caused by Cr in *in vitro* systems. The fact that Cr is essential also implies that oral Cr(VI) could supply the necessary Cr, again implying a threshold at nontoxic doses. Also, experiments from the Costa laboratory (Davidson et al.,Toxicol. Appl. Pharmacol. 196:431-437, 2004; Uddin et al., Toxicol. Appl. Pharmacol. 221:329-338, 2007) showing that chromate in drinking water is a cocarcinogen with solar UV, and the implications of this finding, are not discussed.

Other problems (by page):

Page 176, top of the page, is a good example of the confusion between mutagenicity and other endpoints. The statement "Hexavalent chromium-induced mutagenicity has been demonstrated following oral exposure" is misleading. There is only one mutagenicity assay showing positive effects on eyespots (presumed deletions) in offspring of female rats given drinking water with 62.5 mg Cr(6)/L. The deletions were not confirmed, so the eyespots might be epigenetic events (as Klein found with so-called mutants). All of the other assays are for non-mutagenic endpoints, and tend to be negative for drinking water exposure, but positive for gavage (a more toxic type of exposure).

Page 178, 4.5.1.2, it is claimed that mutagenicity has been evaluated in humans experimentally exposed to hexavalent chromium. No such studies appear in Table 4-25. The paragraph mistakenly refers to mutagenicity many times.

Page 186: It is not ER (excision repair) that is responsible for removal of bulky lesions, but NER (nucleotide excision repair) that is. Reynolds et al. 2004 is missing in references.

Page 187, bottom. It doesn't make sense that mice given 0 mg/kg Cr(6) should have a significant level of apoptosis (compared to what?).

Page 188: The Dai et al 2009 paper does not measure mutation frequency in human cells.

Toby G. Rossman, Ph.D.

Page 190: 3rd paragraph: The mutational spectrum of chromate is not clear. See the review by Klein referenced above.

Page 202: Key Events, #3: The authors skip from discussing mechanisms of DNA damage by Cr to "overall genomic instability which can lead to mutations if not adequately repaired". Genomic instability can occur as a result of other factors besides DNA damage, and genomic instability is not repaired (DNA damage can be, but the repair often leads to apoptosis). As discussed above, toxic exposure would play a role in the selection of Cr-resistant and/or apoptosis-resistant cells. There is no obvious tie-in here between DNA damage and mutagenesis as a key event, since cells resistant to Cr could have arisen by epigenetic silencing (and this may also be a mechanism in resistance to apoptosis). In a sense, this point is recognized in #4, but in postulating apoptosis as a key event, the authors do not seem to realize the implications, i.e. that a toxic dose is needed for carcinogenicity. They suggest that selection for resistance to apoptosis is due to mutations (either pre-existing or Cr-induced) but there is no evidence for this. Besides altered DNA methylation, other mechanisms for the appearance of Cr(VI)-resistance in exposed cells include epigenetic effects via altered histone modification (Sun et al., Toxicol. Appl. Pharmacol. 237:258-266, 2009) as well as reduction of Cr(VI) transport via down-regulation of sulfate ion transporter activity, and resistance to apoptosis via altered gene expression (upregulation of survival pathways and down-regulation of apoptotic pathways) (discussed in Nickens et al., 2010)

p. 204-210: What is described as "mutagenicity" is not in all cases.

p. 206, end of paragraph 1: It is claimed that there is evidence that Cr(6) induces mutagenicity in tissues at the site of entry and systemically at doses relevant to human exposure. Where is the evidence for this?

p. 206, bottom: Again, it is important to note that DNA damage can lead to apoptosis as well as mutation, so it does not necessarily support a mutagenic MOA.

p. 207: De Flora et al., 2008, did not look for mutagenesis, they looked for DNA damage.

p. 208: Neither O'Brien et al., 2005 nor Eastmond et al., 2008 is in references.

p. 209: Low (non-toxic) concentrations would not provide selective pressure for Cr-resistant, mismatch repair deficient cells. Especially in vivo, toxicity would be the driving force to stimulate the outgrowth of such cells.

p. 209, 2nd. paragraph: This is a lot of speculation (should be, may be).

p. 211, bottom: It is claimed that the study by NTP found no evidence of tissue damage or necrosis. Did they look for apoptosis? It is also claimed that most available studies found Cr-induced genetic damage at doses below those that inducing cytotoxicity. This has not been demonstrated, since clonal survival (the only assay that will detect delayed toxicity) was not performed in these studies.

p. 212, end: "...giving rise to mutagenicity (including DNA adduct formation, DNA damage, gene mutations, chromosomal aberrations and micronuclei formation" This is nonsense, as is the next sentence. There is no evidence for mutagenicity at the target tissue at all.

p. 213: More confusion between mutagenicity and other endpoints. It is not true that other hypothesized MOA's have not been demonstrated. There is actually more tumor evidence for an epigenetic MOA. Thus, the weight of evidence favors the alternative. This is not a numbers game. Epigenetic studies are relatively new, compared with DNA damage and other "genotoxic" studies, so there are fewer studies.

p. 214 top: Has EPA concluded this? Then what is the purpose of reviewing this document?

p. 238, bottom: More confounding of mutagenesis and other endpoints.

Additional papers showing epigenetic effects of Cr

Schnekenburger et al., (2007) Chromium cross-links histone deacetylase1-DNA methyltransferase1 complexes to chromatin, inhibiting histone remodeling marks critical for transcriptional activation. Mol. Cell Biol. 27(20): 7089-101.

Sun et al. (2011) Comparison of gene expression profiles in chromate-transformed BEAS-2B cells. PloS ONE 6(3): e17982. Doi:10.1371/journal.pone.0017982

Ali et al. (2011) Aberrant DNA methylation of some tumor suppressor genes in lung cancers from workers with chromate exposure. Mol. Carcinogenesis 50(2):88-99

B3. A two-year drinking water study in rats and mice (NTP, 2008) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantification is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

I do not think that a linear no threshold approach is valid.

B4. The incidence of adenomas and carcinomas combined in the small intestine of male mice from the NTP (2008) two-year drinking water study were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

This seems to be the only choice.

B5. The oral slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk of tumors of the small intestine in male mice). Has the modeling been appropriately conducted and clearly described?

This is outside my field of expertise.

POST-MEETING COMMENTS SUBMITTED BY

Konstantin Salnikow, Ph.D.

Konstantin Salnikow, Ph.D.

Revised comments on the Integrated Risk Information System (IRIS) assessment of hexavalent chromium carcinogenicity.

These comments are part of an external peer-review of the scientific basis supporting the human health assessment of hexavalent chromium that will appear on the U.S. Environmental Protection Agency online database IRIS.

Comments are prepared by Dr. Konstantin Salnikow, Ph.D. Program Director, Division of Cancer Biology, Cancer Cell Biology Branch, National Cancer Institute, NIH.

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazard?

In September 2010, the U.S. Environmental Protection Agency prepared the "Toxicological Review of Hexavalent Chromium" to assess health risks associated with hexavalent chromium exposure. This document will appear on the Agency's online database, the Integrated Risk Information System (IRIS). The existing IRIS file for hexavalent chromium, prepared in 1998, does not consider hexavalent chromium to be carcinogenic by the oral route of exposure. The purpose of the new document is to update the IRIS regarding noncancer and cancer health effects associated with oral exposure to hexavalent chromium after considering the latest scientific evidences.

The prepared draft of the "Toxicological Review of Hexavalent Chromium" provides a detailed analysis of the data obtained in several studies carried out in 2008, 2009 by the National Toxicology Program along with other studies, formulates a mutagenic mode of carcinogenic action, and suggests that the reduction of orally administered hexavalent chromium in guts, even in low doses, is incomplete. The draft also calculates a cancer slope factor for humans. Recognizing the importance and relevance of this document, this reviewer needs to address some shortcomings of the document. In general this is a dense document cataloguing many diverse and sometimes controversial studies in the area of hexavalent chromium toxicology and carcinogenesis. Of course the limitations in available experimental data obtained from existing animal models, ongoing investigations regarding the mode of action (MOA) of hexavalent chromium, and uncertainties in epidemiological data make it difficult to prepare a comprehensive document that will fully address public health concerns.

Chapter 3 should be reorganized and more emphasis should be given to the role of chromium III in toxico- and pharmacokinetics as well as in biological effects produced by hexavalent chromium. Hexavalent chromium is generally considered a much more potent mutagen and carcinogen than trivalent chromium. Lack of carcinogenic effects observed with trivalent chromium compounds can be explained by poor permeability of cell membrane for this ion. <u>However, considering that the end product of intracellular reduction of hexavalent chromium is trivalent chromium, which may accumulate in tissues, it is important to consider what role intracellularly deposited trivalent chromium may play in chromium toxicity and carcinogenesis.</u> The majority of studies indicate that after intracellular reduction of hexavalent chromium to trivalent chromium, it can form various damaging DNA adducts. These adducts can inhibit the enzymatic activity of DNA polymerases, simultaneously increasing the rate of replication and the processivity of the DNA polymerase, and thereby decreasing its fidelity and causing more frequent errors. The frequency of errors increases with a dose-dependent increase in mutation frequency *in vitro* (Snow, 1991; Salnikow and

Zhitkovich, 2008). Unfortunately, it is not clear how applicable these studies are to understanding the effects of trivalent chromium on DNA synthesis and cellular metabolism in vivo because the experiments were done in either in test tubes or in artificial model systems with concentrations far exceeding those obtained through environmental exposure (Snow, 1991; Dai *et al.*, 2009). Numerous attempts have been done to study the distribution and retention of chromium (III) in vivo. Onkelinx studied tissue retention of $^{51}CrCl_3$ in groups of female Wistar rats of various ages (35, 60, and 120 days) after a single intravenous injection of trace amounts of $^{51}CrCl_3$ (Onkelinx, 1977). The study showed that total excretory clearance is the sum of three components: urinary clearance (fu), fecal clearance (fd), and a residual clearance (fs), corresponding to an apparently irreversible deposition of chromium into long term body reservoirs. Consistent with the model, ^{51}Cr was found to accumulate with time in several organs such as bone, kidney, spleen, and liver after a single intravenous injection of $^{51}CrCl_3$. These data are supported by those obtained by O'Flaherty (O'Flaherty, 1996) and others indicating that the retention of chromium III by bone, liver, kidney, and spleen is prolonged.

Also, to make the toxicological review concise, I suggest eliminating Table 4-21"In vitro genotoxicity studies of hexavalent chromium in nonmammalian cells" and Table 4-24 "In vivo genotoxicity studies of hexavalent chromium in *D. melanogaster*" because these are irrelevant to the MOA of hexavalent chromium in mammalian systems.

There are numerous errors throughout the text of the Draft. Some of them are shown at the end of these comments as Errata.

G2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

Key issues that should have an impact on the conclusions of the draft of the Toxicological Review of Hexavalent Chromium are: 1) the use of appropriate animal models, 2) an understanding of the chromium carcinogenic MOA, including genotoxic (mutagenic) and non-genotoxic (epigenetic) mechanisms and their interrelations, 3) the co-carcinogenic effects of hexavalent chromium, and 4) the role of iron metabolism in chromium carcinogenesis. Unfortunately, studies to address these issues are either not done or are in the early stages of research. Thus, it is too early to draw any conclusive decisions on risk assessment of hexavalent chromium in drinking water.

Are used animal models appropriate for risk assessment?

Although the NTP studies provide evidence that oral exposure to hexavalent chromium induced tumors in rodents, the main argument against these studies is that the toxic and carcinogenic effects could be achieved/observed only at high chromium concentrations, which significantly exceed human exposure levels. Also, it is noted that biological effects were seen only at chromium concentrations that overwhelmed the cellular defense systems (reducing capabilities). Ascorbate is a major reducing agent of hexavalent chromium in biological fluids and tissues (see review (Zhitkovich, 2005; Salnikow and Zhitkovich, 2008)). Humans cannot synthesize ascorbate in the body because of a mutation in the L-gulono-γ-lactone oxidase gene coding for the final enzyme in ascorbate metabolism and thus ascorbate is supplemented through the diet. Unlike humans, laboratory mice and rats, used for carcinogenicity assays, are capable of synthesizing ascorbate endogenously. Because ascorbate regulates many cell and tissue functions that are critical for cancer development, the changes in the level of ascorbate should be considered in animal models of choice (Salnikow and Kasprzak, 2005). It is impossible to deplete tissue ascorbate levels by metal exposure in wild-type rodents because the enzyme producing ascorbate will be up-regulated when the level of ascorbate drops below a critical point. To avoid this problem for *in vivo* testing of the toxic and carcinogenic effects of heavy metals, which efficiently destroy ascorbate, an appropriate model is the use of mice or rats that like humans

cannot synthesize ascorbate (Kasprzak *et al.*, 2011). Two rodent model systems unable to synthesize ascorbate are available: Gulo-/- mice (Maeda *et al.*, 2000), and a similar rat strain (Mizushima *et al.*, 1984). Our preliminary data show that when Gulo-/- mice were supplemented with ascorbate in drinking water their blood and tissue ascorbate levels were undistinguishable from that in wild type mice. However, ascorbate levels were significantly decreased by metal exposure in Gulo-/- mice but not in wild-type mice, in which the enzyme responsible for ascorbate production was activated in response to metal exposure (ascorbate depletion) (Kasprzak *et al.*, 2011). In this model system we found that the reduction in ascorbate levels increased acute toxicity induced by Ni_3S_2 in Gulo-/- mice and that Gulo-/- mice were more susceptible than wild-type mice to nickel-induced carcinogenesis. Additionally, in tumor transplantation assays, Gulo-/- mice had shorter tumor latency than wild-type mice. After the lag period established tumor growth rates were comparable in Gulo-/- and wild-type mice. Although cancer initiation and development is a very complicated process our results indicate that ascorbate is a potentially important part of the molecular mechanisms of metal carcinogenesis and acute toxicity.

Ascorbate is involved in diverse biological activities. Ascorbate is essential for the function of numerous 2-oxoglutarate-dependent hydroxylases. This group of hydroxylases includes the asparaginyl and prolyl hydroxylases, FIH-1 and PHD1, PHD2, PHD3, which are responsible for HIFα hydroxylation (Epstein *et al.*, 2001; Mahon *et al.*, 2001; Hewitson *et al.*, 2002; Lando *et al.*, 2002); the collagen prolyl-4-hydroxylases (Myllyharju, 2003), which is critical for extracellular matrix formation; and a new class of histone and DNA demethylases that remove methyl group through hydroxylation (Shi, 2007). We already pointed out that the level of ascorbate is critical for metal carcinogenesis mainly by affecting epigenetic pathway (Salnikow and Zhitkovich, 2008). More recently a link between changes in ascorbate concentration and DNA demethylation of human embryonic stem cells (hESCs) has been identified (Chung *et al.*, 2010). Thus, ascorbate levels have the potential to directly impact the differentiation of hESCs and the reprogramming of somatic cells.

Given that ascorbate has diverse cellular functions and ascorbate levels are critical to interpreting carcinogenic effects of heavy metals, the animal models described in the Toxicological Review are not the most appropriate. The results obtained in NTP 2007 studies are consistent with the idea that ascorbate is an important factor to consider. In preliminary toxicokinetic studies in which animals were exposed to chromium in drinking water for 21 days chromium concentrations in the blood of the guinea pigs (which are unable to synthesis ascorbate) was greater than chromium concentrations in the blood of the rats or mice suggesting greater absorption (less reduction) of chromium in guinea pigs.
http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox072.pdf

I suggest that new studies similar to the NTP studies with several dietary concentrations of ascorbate and lower chromium does (i.e., those more relevant to environmental exposures), be done in ascorbate-deficient rats or mice or both animal models. Additionally, because of more efficient depletion of ascorbate in tissues of Gulo-/- animals by chromium these animal models will show whether Cr(III) and Cr(VI) kinetic that were developed using the wild type animals (O'Flaherty, 1996; O'Flaherty *et al.*, 2001) will be applicable to Gulo-/- animals. This will allow for adjustment of kinetic models, if needed, and identification of new or confirming known compartments of chromium retention.

A1-A4 Oral Reference Dose (RfD) for Hexavalent Chromium

Outside of my area of expertise

Konstantin Salnikow, Ph.D.

Carcinogenicity of Hexavalent Chromium

B1.

Hexavalent chromium has been classified by IARC as carcinogenic to humans (group 1) via inhalation route of exposure based on results obtained in human and animal studies. However, when animals were exposed to hexavalent chromium in drinking water the carcinogenic effects were observed only at very high doses, which are irrelevant to human exposure. These results seems to cast doubt on carcinogenicity of hexavalent chromium via oral route of exposure and yet as I already pointed out in G2 the reason for only high chromium doses producing carcinogenic effect may be stemming from the inappropriate animal models which have higher protective ascorbate levels as compared to humans. Although, more human and animal studies are required to make an informed conclusion the ability of hexavalent chromium to produce tumors makes it likely to be carcinogenic by oral exposure.

Another important consideration is that hexavalent chromium is a co-carcinogen and consumption of water with other toxic or carcinogenic compounds will result in unraveling chromium carcinogenic effects at much lower doses. Additionally, people with chronic inflammation of digestive tract could be more susceptible to chromium-induced carcinogenesis.

B2.

MOA. Genotoxic effect of hexavalent chromium.

The draft of the Toxicological review provides a substantial body of information regarding the mutagenic potential of hexavalent chromium and concludes that hexavalent chromium is carcinogenic by a mutagenic MOA. Indeed this topic has been studied extensively. The results of in vitro and in vivo studies provide substantial evidence for the mutagenic activity of hexavalent chromium, which is mediated through the generation of the highly reactive chromium intermediates penta- and tetravalent chromium, reactive oxygen species, and trivalent chromium formed during the intracellular reduction of hexavalent chromium. These chromium and oxygen species can react with DNA, leading to oxidative DNA damage, chromium-DNA adducts, DNA strand breaks, and chromosomal aberrations. Despite these studies, the significance of chromium-induced DNA damage in the mechanisms of chromium carcinogenicity is not clear. If DNA damage/mutations are important and a causative factor in chromium-induced carcinogenesis, then these mutations should be frequent in chromium-induced tumors. However, very few publications exist in this respect. In animal studies, De Flora et al. (De Flora *et al.*, 2008) found no evidence of DNA-protein crosslinks and DNA adducts in the duodenum following drinking water chromium exposures. Other available hexavalent chromium drinking water exposure studies that measured mutagenicity in mice also could not detect evidence of micronucleus induction in the blood or bone marrow (Mirsalis *et al.*, 1996; De Flora *et al.*, 2006; De Flora *et al.*, 2008). Analysis of lung cancers from chromate-exposed workers revealed that p53 mutations are not very frequent, with only six missense mutations identified in 4 (20%) of the 20 chromate lung cancer samples (Kondo *et al.*, 1997). There were fewer mutations in the patients with lung cancers who had been exposed to chromate than in those who had not (20% vs about 50%). This study also revealed that there was no association between p53 mutations and the period spent working in chromate factories. It is conceivable that chromium causes genotoxic effects, damage DNA, but this damage is efficiently repaired and do not play any role in carcinogenic effects of chromium.

Thus, in order to confirm or refute a possible role of chromium genotoxic/mutagenic effects in chromium-induced carcinogenesis, comprehensive analyses of mutations in oncogenes and tumor suppressor genes in experimentally induced tumors in animals should be done first, followed by more detailed sequence analyses

of chromium-exposed human tumors. A feasible study that could be done in a short period of time (assuming that tumor samples collected in the NTP studies are stored and frozen) is **exon only global sequencing** of DNA from chromium-induced rat tumors versus spontaneous tumors (several spontaneous tumors of different origin were observed in NTP 2008 study). These studies will allow comparison of tumor driving mutations versus passenger mutations.

MOA, Epigenetic effects of hexavalent chromium.

Global changes in the epigenetic landscape are a hallmark of cancer. The initiation and progression of cancer, traditionally seen as a genetic disease, is now realized to involve epigenetic abnormalities along with genetic alterations. Recent advancements in the rapidly evolving field of cancer epigenetics have shown extensive reprogramming of every component of the epigenetic machinery in cancer including DNA methylation, histone modifications, nucleosome positioning and non-coding RNAs, specifically microRNA expression (Sharma *et al.*, 2010). Epigenetic effects have also been observed following hexavalent chromium exposure (Salnikow and Zhitkovich, 2008; Arita and Costa, 2009). Increased DNA methylation was observed in the promoter region of the tumor suppressor gene p16 and the MMR gene hMLH1, indicating that chromium can induce epigenetic effects (Takahashi *et al.*, 2005; Kondo *et al.*, 2006). Gene transcription has also been shown to be affected by exposure to hexavalent chromium in vitro via epigenetic mechanisms. Sun et al. (Sun *et al.*, 2009) found alterations in the levels of histone methylation in human lung A549 cells exposed to hexavalent chromium, indicating the capability of these exposures to lead directly to changes in gene expression. Taken together, these studies suggest that epigenetic mechanisms may contribute to the carcinogenicity of hexavalent chromium. However, it is not clear whether epigenetic changes produced by chromium exposure are acting alone or linked to chromium genotoxic effects and that both genetic and epigenetic changes are essential for tumor appearance and evolution. More research is needed in this area including the identification of changes in DNA methylation (analyses of frequency of inherited silencing of tumor suppressors in tumors and in miRNA expression patterns following chromium exposure in animal models and humans before any conclusions can be drawn regarding the role of epigenetics in the carcinogenic effects of hexavalent chromium.

MOA. Co-carcinogenic effects of chromium.

Co-carcinogenic effects of hexavalent chromium were reviewed recently (Salnikow and Zhitkovich, 2008). The majority of occupational and probably all environmental exposures to hexavalent chromium occur as co-exposures with other carcinogens. The most common examples of co-exposures occur among stainless steel welders, and among hexavalent chromium-exposed workers who are also smokers. Two reports from the Costa Lab (Davidson *et al.*, 2004; Uddin *et al.*, 2007) provided strong experimental data demonstrating that hexavalent chromium can act as a potent co-carcinogen for UV-induced skin tumors. In both studies, the presence of hexavalent chromium in drinking water caused dose-dependent increases in the frequency of skin tumors in UV-irradiated hairless mice. Hexavalent chromium alone produced no tumors, indicating that it acted a strong enhancer of UV-initiated tumorigenesis. Supplementation with vitamin E or selenomethionine had no effect on hexavalent chromium-mediated enhancement of skin carcinogenesis suggesting that co-carcinogenic effects were not oxidant-mediated. It is noteworthy that the level of chromium in skin directly exposed to UV had significantly higher levels of chromium than underbelly skin that was not directly exposed to UV in mice exposed to UV and 5 ppm K_2CrO_4 ($P < 0.05$) (Davidson *et al.*, 2004). This raises an interesting question, does inflammation, whatever the source, facilitate chromium accumulation or delay chromium clearance? This is an important and understudied area. The identified co-carcinogenic effects of hexavalent chromium raise an intriguing possibility that much lower doses of chromium could be hazardous

under certain circumstances when exposure to chromium in drinking water is combined with other harmful exposures.

Another important area of research is an understanding of the role of Inflammation/colitis in hexavalent chromium carcinogenesis. It is well know that at least 20% of all cancers arise in association with infection and chronic inflammation and even those cancers that do not develop as a consequence of chronic inflammation, exhibit extensive inflammatory infiltrates with high levels of cytokine expression in the tumor microenvironment. Aberrant activation of NF-κB and/or STAT3 is found in over 50% of all cancers and renders premalignant and fully transformed cells resistant to apoptosis and speeds up their rate of proliferation, thereby increasing tumor growth. It is extremely important to test whether hexavalent chromium will be more carcinogenic and at lower doses in animals in which colitis was induced, for example by sodium dextran sulfate.

MOA. Interference with iron metabolism.

In rats neoplastic changes were found at sites of tissue contacts with the highest concentrations of hexavalent chromium, i.e. oral cavity. This may be explained by the immediate damaging effects of chromium on DNA and other cellular components. At the same time frequent nonneoplastic changes were observed in duodenum of male and female rats and neoplastic changes were observed in duodenum of male and female mice. These data cannot be explained by the direct effect of hexavalent chromium, which should be mostly reduced by the time it reaches small intestine. Considering that in other organs such as liver and kidney which accumulate significant amount of chromium no tumors were observed, it is important to do more research on the mechanism of tumor development in small intestine. Specifically, duodenum is a place where iron absorption takes place. An analysis of ferroreductase expression and iron metabolism will help to shed light on whether an alteration in iron metabolism in this tissue may be responsible for chromium carcinogenic effects.

B3.

Outside of my area of expertise.

B4.

It seems that combining the incidence of adenomas and carcinomas in small intestine was the proper choice for modeling cancer risk. This is supported by the available data and clearly described. The fact that only highest doses produced a carcinogenic effect may indicate high reducing capacity in tested model systems (wild type mice and rats). As suggested in A2 exposing Gulo-/- mice or rats to hexavalent chromium may result in tumor appearance at lower doses. If this will be the case the extrapolation to environmentally relevant doses of chromium exposure will be more feasible.

B5.

Outside of my area of expertise.

Errata:

Regarding the form of the draft it should be noted that, although this is not a manuscript, it is necessary to correct errors and mistakes in the draft content.

Below are several examples:

1. **$K_2Cr_2O_4$** – does not exist (page 30, table 3-7; page 45), **should it be K_2CrO_4**
2. Table 2-1, page 6 – **Cr_2O_3** is chromium (III), not hexavalent chromium. **$BaCrO_4$** is barium chromate, not barium oxide.
3. Table 2-5, page 18 – "accumulates Cr(V)", the intent of this statement is not clear because this form is short living and unlikely that is can accumulate.
4. Page 35 and Table 3-9, **K_2CrO_7** - does not exist
5. None existing or wrong citations:
   Kumulainen, 1991 (page 7), **should be Kumpulainen, 1992** (page 251).
   Salnikov and Zhitkovich, 2009 (page 50); Salnikov and Zhitkovich, 2008 (page 257), **should be Salnikow and Zhitkovich, 2008**.
   Costa and Klein, 2004 (page 66); Costa, M.; Klein, C.B. 2008 (page 245), **should be Costa and Klein, 2006**.
   LeVina et al., 2003, 2007, (page 50) **should be Levina et al., 2003, 2007**.
   Kasprzak, 1996 (page 189), **no reference provided.**
   Campbell J.L.; Tan,Y.; Clewell, H.J. (2009) Development of a PBPK model for hexavalent chromium in rats and mice to estimate exposure to oral mucosa and small intestine. Toxicologist 108(1):98 (Abstract) Poster ID # 108. This is a **questionable citation**.
   Sun et al. (2009), (page 188), **no reference provided.**
   Davies, JM. (1979) Lung cancer mortality of workers in chromate pigment manufacture: An epidemiological survey. J Oil Chem Assoc 62:157-163, (page 245), **should be: J Oil Colour Chem Assoc 62:157-163**

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POST-MEETING COMMENTS SUBMITTED BY

John Pierce Wise Sr., Ph.D.

John Pierce Wise Sr., Ph.D.

Toxicological Review of Hexavalent Chromium

PRE-MEETING WRITTEN COMMENTS

CHARGE QUESTIONS

General Charge Questions:

G1. ***Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazard?***

The Toxicological Review is logical, clear and concise. However, overall the document is inconsistent and thus, EPA has not presented and synthesized the scientific evidence for noncancer and cancer hazard in a clear manner. Some sections, primarily the ones focused on animal data are clearly presented and synthesized. These sections present the primary literature and discuss the merits of each study with balance and insight.

Other sections, however, particularly those involving *in vitro* cell culture data and underpinning the mode of action are much less appropriately considered, are not well-presented, and do not synthesize the underlying data very well. Determining a mode of action is a key part of the risk assessment. The Toxicological Review would be a stronger document if it fully analyzed and synthesized the primary literature to ascertain the possible modes of action for Cr(VI). The strengths, weaknesses and data gaps for each could have been highlighted and discussed, and then a rationale for the chosen mode of action presented. However, as presented this approach is not apparent.

Instead, as presented, the document gives the impression that the mode of action was pre-determined from a select set of review articles and the best case for that mode of action presented. Decisions appear to have been made to agglomerate all of the genotoxicity data into positive or negative proof of mutagenesis rather than more careful consideration of individual lesions. Confounding factors, such as ascorbate levels, are cautioned against, but inadequately and inaccurately presented and unevenly applied, which undermines confidence that the primary data were adequately considered and contributes to a perception that decisions were predetermined. This perception is strongly reinforced by poor management and citation of the underlying literature and a heavy reliance on a few select review articles that unfortunately miscite the primary literature. As a result, many sections of the Toxicological Review lack clarity, accuracy, synthesis and rigor and the rationale for the choice of mode of action seems predetermined and forced. Each of these factors is elaborated on in more detail below.

John Pierce Wise Sr., Ph.D.

1. Unnecessary agglomeration of genotoxicity data

The Toxicological Review essentially combines all of the lesions related to genotoxicity (stated on page 212 as "*...including DNA adduct formation, DNA damage, gene mutations, chromosomal aberrations, and micronuclei formation*") into one bundle and refers to them as "mutagenicity". This decision is typically based on the presumption that the various genotoxic lesions will ultimately manifest as mutations in the primary DNA sequence. Hence, it is generally recognized that a crosslink or a strand break is not inherently a mutation, but that it may eventually manifest as one and thus, it is a mutagenic event. Based on the aggregation of all of these data, the Toxicological Profile declares Cr(VI) to be a mutagen and proposed a mutagenic mode of action.

This approach is consistent with older practice and perception of these genotoxicity assays and it may be a useful approach for a chemical with a limited data set. However, the genotoxicity data for Cr(VI) is a rich data set and deserves a more sophisticated consideration. The Cr(VI) literature often distinguishes in its presentation between mutagenic and genotoxic lesions. The Toxicological Review does not carry forward that distinction and does not explain the rationale for ignoring it. However, it is an important distinction because not all of these lesions are likely to be mutagenic after Cr(VI) exposure. Aggregating these lesions oversimplifies the interpretation of the data and masks the fact that much of the primary data suggest that in actuality, Cr(VI) is a very weak mutagen. Discussing each class of lesion on its own merit with a more careful consideration of the primary literature would have better framed the strengths and limitations of the genotoxicity studies and brought this discussion into a clearer light.

The fundamental problem underlying this section is a failure to clearly consider the primary literature to see that Cr(VI) is a weak mutagen when defined as an agent that can directly change the primary DNA sequence. Cr(VI)-induced mutations have indeed been observed in bacteria, cultured cells and animal studies. However, in most cases, one has to experimentally force the mutations to occur by using a high dose, a forced experimental system or a non-physiological exposure route.

There is no real synthesis of this literature beyond listing outcomes in a table. The Toxicological Review also considers the results as simply positive or negative. That certainly is one approach; however, it misses the opportunity to consider the data more thoroughly. Some consideration should be given to potency and its potential impact and the robustness of the underlying assays. If the experimental data show that Cr(VI) induces a 2-fold increase in mutations, then the Toxicological Review would call that outcome positive. However, if in that same assay an established mutagen induced a 50-fold increase in mutations, then does Cr(VI) still appear to be a mutagen? Or does it suggest a different mode of action, particularly when the frequency of mutations have not been reported to increase in Cr(VI)-induced human tumors? Does the fact

that ascorbate is higher in rodents make these mutations a rodent-specific event? A more careful and thoughtful presentation of the underlying data would have better informed the consideration of this mode of action.

In addition, before lumping all of these genotoxic endpoints together as all mutagenic outcomes, a careful review and discussion of the primary mutagenesis literature is needed. That review needs to determine, for Cr(VI), which of the various lesions (e.g. DNA adducts, DNA crosslinks, DNA strand breaks, gene mutations, chromosome damage, etc.) actually occur in cells (for example as discussed below the adducts may not actually form in cells) and to what extent they occur. Then, the review needs to determine which, if any, of these lesions actually lead to gene mutations. The discussion below illustrates that there are reasons to doubt that the various lesions are all mutagenic outcomes. After this analysis, those lesions that do form in cells and that do produce mutations could more reasonably be combined into a category of "mutagenicity".

Perhaps, the data will indicate Cr(VI) is a mutagen, but, perhaps, the data indicate that one only gets mutations in the DNA sequence when systems are forced experimentally to do so at very high concentrations, due to species specific factors or by non-physiological exposure routes. If the latter were true, this possibility would suggest that mutations are not likely to occur in humans, raising direct implications for the mode of action decision. A more thorough treatment of the primary mutation data is needed to clarify these important points.

There is concern that the discussion of some lesions is overstated while others are mentioned but not discussed. The section explaining DNA adducts is greatly overstated and also does not fully consider the primary literature. The section presents a case that implies the status and impact of the various potential adducts are known in cells and *in vivo*. The Toxicological Review even provides a structure of a Cr-DNA adduct. The major concern is that when the primary literature is fully considered, it becomes apparent that these adducts are all based on cell-free systems and no one has been able to clearly identify any specific adducts in cells, whole animals or humans beyond observing tangles of DNA, protein, and Cr that are considered to be DNA-DNA or DNA-protein crosslinks. The primary literature has only measured adduct levels in cells by isolating DNA and then measuring the amount of Cr associated with it or by nonspecific P32 postlabelling. These measures cannot ascertain how or if Cr is bound to the DNA, only that it is associated with it in some way. Some studies have synthesized adducts in cell free systems and applied them to cells, but that does not mean those specific adducts form in the cell.

Thus, it is unknown if specific DNA adduct events occur in cells, whole animals or humans. Nonspecific adducts have been detected by postlabelling, but specific adducts remain elusive. To lump these studies together as clear evidence of mutagenicity gives them a weight of evidence that seems premature and inaccurate. Overall, this section is very misleading in its portrayal of the status of adducts as more understood than they actually are.

Oxidative damage is also included as evidence of mutagenicity. However, discussion is missing to establish whether this oxidative damage is a direct effect of Cr(VI) causing oxidative damage to DNA and thus, potentially a mutagenic event, or if this damage is actually indirect, resulting from overall oxidative stress to cells caused by high doses of Cr(VI) depleting intracellular antioxidants.

Cr(VI)-induced strand breaks are cited as another type of mutagenic event. These lesions are discussed as post-replication-induced breaks. However, the discussion fails to question and discuss whether or not these are actually frank DNA breaks. As the Toxicological Review indicates, the studies have focused on gamma-H2A.X focus production as the measure of breaks. Data indicate that chromatin remodeling may also induce the production of gamma-H2A.X so they may not be frank DNA breaks after all. This possible explanation is missing from the section.

DNA-protein crosslinks are included as a type of mutagenicity in the tables and description despite the fact that the document states on page 186 that it is unknown if they are mutagenic: "*Tests for the mutagenicity of these crosslinks have proved inconclusive (reviewed in Macfie et al., 2010), but the bulkiness of these lesions indicates the potential for genotoxicity...*"

The most consistent genotoxic outcome is the production of damage to metaphase chromosomes manifested as aberrations, sister chromatid exchanges, and micronuclei. The fact that Cr(VI) induces these events was presented but not discussed. This lesion may be the key lesion as it is the most consistent and yet the mechanisms that may underlie it are ignored and not discussed. Chromosome damage could be a mutagenic lesion as assumed. Alternatively, it could be the consequence of epigenetic changes in the cell resulting from Cr binding to centrosomes in the mitotic spindle assembly apparatus or from bypass of the spindle assembly checkpoint. Cr(VI) has been shown to affect centrosomes and the spindle assembly checkpoint and perhaps it causes uneven pulling leading to breaks and errors in chromosome number. Cells with broken chromosomes may undergo apoptosis, while those with increased chromosome number may go on and survive as highly aneuploid cells. Cr(VI) has been shown to induce highly aneuploid cells that can clonally expand and survive. This outcome would not be consistent with a mutagenic mode of action.

These concerns above are only magnified by the problems with uneven consideration of confounders and in poor management of the underlying literature described below. Together these factors give the impression of excluding or avoiding different syntheses of the data. More care and balance are needed to discuss and consider the genotoxicity data separately and evaluate if they are mutagenic markers.

2. Uneven application of experimental confounders

The perception of bias caused by bundling all of the genotoxicity endpoints together is magnified by an apparent uneven consideration of experimental confounders in the document. It appears that the Toxicological Review does not fully consider and present all of the relevant *in vitro* cell culture data that inform possible modes of action. Instead, selected examples of primary literature that reinforce one point of view are presented. This approach undermines the synthesis of the literature and because of a marked unevenness in presentation creates a perception of bias that should not be part of the analysis.

For example, on page 184-185, the Toxicological Review gives the reader the impression that ascorbate-trivalent chromium-DNA adducts have been found to be highly mutagenic. However, the section did not describe the experimental detail that indicate the cells used in the study were abnormal and genetically modified so that they could not carry out proficient DNA repair or apoptosis, or that the cells were not actually treated directly with Cr(VI). These omissions stand in stark contrast to the experimental criticisms the Toxicological Review applies to other cell culture studies.

For example, on page 47, the document states:

> "*Caution should be used in interpreting cell culture data, as the cell culture medium could play a role in hexavalent chromium reduction, confounding the extent of intracellular hexavalent chromium reduction.*"

It then cites a couple of examples where Cr(V) was detected in extracellular cell culture medium. The use of the word "caution" leads the reader to conclude that many *in vitro* studies may be flawed due to this reduction. No explanation is offered as to why this outcome is a problem. No discussion is provided that points out whether this same type of reduction might be expected to occur outside of cells in the body and thus actually be normal. The practical reality is that reducing agents are present in the extracellular fluid and thus, some extracellular reduction probably occurs in the extracellular fluid. This factor is probably not appropriate as a cautionary one and may actually reflect physiological conditions. But, no balanced discussion is provided for the reader to decide if this factor is indeed a concern.

A similar unevenness occurs during the presentation of the relative importance of ascorbate. The Toxicological Review states on page 50:

> *"An additional important note on these biotransformations regards the interpretation and reliability of data from in vitro assays. In vivo, the intracellular levels of ascorbate are quite high (about 1 mM). In contrast, the levels of ascorbate in tissue culture media are quite low since generally it is not added to the media so that the only source is supplemented fetal bovine serum (FBS). With 10% FBS, the level of ascorbate in tissue cultured cells is only about 50 μM which is 20 times lower than that which is found in vivo (Zhitkovich, 2005). Therefore, experiments on mutagenesis and other toxic effects of hexavalent chromium in tissue culture may underestimate its mutagenic, genotoxic, and cell-transforming activities (Zhitkovich, 2005; Costa and Klein, 2006)."*

No further discussion is presented. There is no presentation of the primary literature in cell culture showing the impact or lack of impact of this difference, just speculation that it might cause some underestimation. There is no presentation of the primary literature to establish what the ascorbate levels are in the relevant tissues of concern. There is just this one review article (Zhitkovich 2005) with some comment from a secondary review article (Costa and Klein 2006) cited. Closer inspection shows that the Costa and Klein review is actually citing the same Zhitkovich review so in the final consideration, this entire section relies only on Zhitkovich 2005 which, as discussed below in section 3.D., miscites the primary and secondary literature and draws a conclusion the primary literature does not closely support.

Thus, the Toxicological Review draws attention to the possible presence of extracellular metabolism and lack of intracellular ascorbate as confounding factors, which it may have used to exclude some cell culture studies. But, by contrast, it makes no mention and expresses no concern about studies done in abnormal compromised cells treated only indirectly with Cr(VI). This discrepancy makes the document and its treatment of the underlying literature seem uneven.

Moreover, in its discussion of the impact of ascorbate, the Toxicological Review does not discuss the potential impact of ascorbate differences on the bacterial mutation studies or the possible impact of ascorbate differences on the animal genotoxicity data. For example, in Table 4-23 on page 172, the Toxicological Review indicates a positive effect for mutagenicity in mice after intratracheal instillation. Mice, however, have more ascorbate in their lung tissue. If one accepts the speculation in the Toxicological Review that ascorbate–trivalent chromium–DNA adducts form and are highly mutagenic, then the elevated mutations in this study might simply be due to the elevated ascorbate levels in this species suggesting a species specific effect. Such a possibility would explain why there are mutations in rodents but not in human tumors.

Regardless of which conclusion is correct, the point is that the Toxicological Review does not appear to apply this confounder it stresses in the *in vitro* work evenly to all studies reinforcing a perception of selective bias.

Similarly, the Toxicological Review presents the primary research studies by Quievryn et al., 2003; Voitkun et al., 1998 as showing adduct effects, but both studies used the cell culture medium the Toxicological Review expresses concerns about and neither study addressed the ascorbate concern, but these aspects are not mentioned in the document. The absence of discussion of these confounders in these experiments give the impression that the Toxicological Review does not seem to apply its confounding criticisms evenly.

This inconsistent presentation of experimental expectations and application of confounding factors creates a perception of uneven evaluation of the primary literature. Considered together, the language and approach suggest a strong bias against *in vitro* studies and the cautionary language should be removed to eliminate that bias.

The discussion about ascorbate needs to be more balanced and thorough and the information better synthesized. The ascorbate section could be removed or if the EPA feels the issue needs to be considered, it should be fully vetted with a discussion of how differences in ascorbate might affect the interpretation of the bacterial mutagenesis studies and the rodent data. The discussion would need to also include the strengths and limitations of the primary literature. The relative merits of data from a primary normal human cell line without vitamin C supplementation versus data from a tumor-derived cell line with ascorbate supplementation would need to be presented and discussed. The various underlying phenotypic issues in cell lines would also need to be considered as a mitigating factor. Similarly, the technical limitations of ascorbate supplementation in culture would need to be considered including how long it is retained by the cell and the impact of its diffusion out of the cell and into the extracellular medium.

This discussion would need to include a full evaluation of ascorbate levels in tissues of interest in humans and animals and whether those levels are intracellular or extracellular or both. It should include a full discussion of any data that show whether or not there is an actual impact of different ascorbate levels on outcomes inside the cell from cell culture studies. It should also include a discussion about the fact that ascorbate in the cell becomes depleted over time after Cr(VI) exposure and whether the relevant exposure is when ascorbate levels are normal or depleted.

3. Poor management and citation of the literature

The above two concerns are further magnified by the presentation and management of the literature in the sections involving *in vitro* cell culture data and underpinning the mode of action. There is a tendency in the document to overstate the findings of the selected primary literature included in the document, raising questions about whether the primary literature was properly evaluated and weighed. There is inconsistent application of the phrases "*in vitro*" and "*in vivo*" resulting in substantial confusion regarding the underlying literature and reinforcing a perception of inaccuracy in the document. There are flaws in citations, extensive direct quoting and long stretches of general paraphrasing of a small number of review articles raising questions about the heavy reliance on those articles and points of view. Finally, there is often a failure to check the underlying primary research studies cited in these review articles reinforcing the perception that the document relies on the review and not the underlying primary research data. Considered together, these aspects raise questions about the process of how the conclusions were drawn, create confusion about whether the primary data were fully reviewed or whether the view of the authors of those few review articles was simply adopted, and raise significant questions about the credibility of the overall evaluation. Each concern is explained in more detail below.

A. Overstating the findings of the selected primary literature.

There are concerns that the Toxicological Review over-generalizes its presentation of the primary literature, particularly with respect to *in vitro* cell culture studies. One example of this problem is seen in its discussion of the literature concerning DNA adducts where the Toxicological Review states on pages 184-185 that:

> "*Although the ascorbate–trivalent chromium–DNA adducts are recovered less frequently in vitro due to the low concentrations of vitamin C present in commonly used tissue culture media (Zhitkovich, 2005), these adducts have been shown to be the most mutagenic of all the ternary adducts (Quievryn et al., 2003).*" ... "*They have been detected in vitro in Chinese hamster ovary cells following exposure to hexavalent chromium, and account for up to 50% of all chromium–DNA adducts. The ternary adducts have been found to cause mutagenic and replication-blocking lesions in human fibroblasts in vitro (Quievryn et al., 2003; Voitkun et al., 1998).*"

Thus, the reader is led to believe that ascorbate-trivalent chromium-DNA adducts have been found in cells and these adducts have been shown to be highly mutagenic. Careful examination of the two cited references reveals that the statement in the Toxicological Review quote listed above that states:

> "*They have been detected in vitro in Chinese hamster ovary cells following exposure to hexavalent chromium...*"

is incorrect. Detection of adducts in Chinese hamster ovary cells was not actually presented as data in either paper or mentioned in the text of either paper. The claim is unsubstantiated as presented, which makes it potentially misleading.

Furthermore, when one considers the experimental detail in Quievryn et al., 2003 and Voitkun et al,, 1998, one learns that the adducts were synthesized in a cell free system. A sequence of DNA was treated with Cr(VI) and ascorbate in a cell free system. Then, the damaged DNA sequence was administered to cells. The cells then converted the damaged DNA sequence to a mutation that was revealed when the sequence was recovered and sequenced.

The detail also shows that the cells used were not normal human cells, but rather a SV40 immortalized cell line. SV40 is known to silence p53 activity, among other cellular and molecular changes, thus these cells were unable to carry out proficient DNA repair or apoptosis, as these are normally p53-dependent events.

Thus, the studies did not show that these adducts were normally present or able to form inside the cell. They could not account for the fate of the adduct structure after the transfection process and are only assuming it remained intact. The studies did not show that mutations would have occurred normally inside the cell as a consequence of Cr(VI) exposure or as a consequence of these lesions. Moreover, they do not show that these events would have happened in a repair proficient or apoptosis-proficient cell. It could be that the only reason mutations were seen is that the cells' ability to repair or eliminate them through apoptosis was artificially turned off beforehand.

An alternative interpretation of the studies could be that one can experimentally force a cell to generate a mutation in response to a Cr adduct if repair and apoptosis are silenced. Indeed, a step forward, but not one that establishes that adducts form or are mutagenic in cells.

It is unclear why these two studies were chosen to show that Cr induces adducts in cells. It is remarkable that given the emphasis the Toxicological Review places on the importance of physiologically relevant cell cultures, that it would fail to mention or discuss the integrity of the cell line itself, which in these studies were not robust cells. The document seems to be saying that there is a problem with cell culture studies that have some extracellular metabolism of Cr(VI) or that might not have enough ascorbate, but there are no problems with studies in cells with compromised DNA repair and cell death pathways.

There are more examples of this type of exaggeration of the implications of the primary literature in the Toxicological Review. These exaggerations obscure the meaning and applicability of the data and should be

corrected. These exaggerations also undermine the integrity of the document and raise questions about its accuracy and process. Other studies in the primary literature may reemerge as more relevant if treated more evenly and these studies should be reconsidered and possibly presented.

B. Flaws in citations, extensive direct quoting and long stretches of general paraphrasing

One concern is that the Toxicological Review actually appears to directly quote sources without indicating the comments are quotes of the original source. For example, in the Toxicological Review on page 46, lines 13-15, the writing states:

> *"Studies on the reduction of Cr(VI) by extracts of rat liver, lung, or kidney have found that ascorbate accounted for at least 80% of Cr(VI) metabolism in these tissues (Standeven et al., 1991,1992)."*

which is the exact same sentence that occurs in the Toxicological Review's Zhitkovich 2005 reference. That reference states on its page 5:

> *"Studies on the reduction of Cr-(VI) by extracts from rat lung, liver, or kidney have found that ascorbate accounted for at least 80% of Cr(VI) metabolism in these target tissues (45, 46)."*

Then on the same page, lines 20-22, the Toxicological Review states:

> *"Depending on the nature of the reducing agent and its concentration, this process can generate various amounts of unstable Cr(V) and Cr(IV) intermediates (Stearns et al., 1994)."*

which is the exact same sentence that is in Zhitkovich 2005. That reference states on its pages 5-6:

> *"Depending on the nature of the reducing agent and its concentration, this process can generate various amounts of unstable Cr(V) and Cr(IV) intermediates (14-16)."*

Neither of these sentences are indicated as being exact quotes of the original source and neither one is attributed to the original source. This omission is a concern as it is important to know when the document is choosing to quote from a source directly. These examples are not the only occurrences of this type of error and the entire document needs to be checked to identify other such problems.

In other instances, the Toxicological Review only changes a couple of words in a direct quote and fails to indicate it is a direct quote, which is also unacceptable. For example, the Toxicological Review states on page 50:

> *"Therefore, experiments on mutagenesis and other toxic effects of hexavalent chromium in tissue culture may underestimate its mutagenic, genotoxic, and cell-transforming activities (Zhitkovich, 2005; Costa and Klein, 2006)."*

The underlying reference by Costa and Klein 2006 states on its page 157:

> *"Thus, experiments on mutagenesis and other toxic effects of hexavalent Cr in tissue culture may underestimate its mutagenic, genotoxic, and cell-transforming activity (Zhitkovich, 2005)."*

The quote in the Toxicological Review and the Costa and Klein review differ by only substituting a "Therefore" for a "Thus" at the beginning and "activities" for "activity" near the end. Changing two words does not avoid the need to offset this sentence as a direct quote. As written, it is sufficiently in the original authors' words that it is considered a direct quote.

Similarly, in the Toxicological Review on page 46, the writing states:

> *"Ascorbate is also the fastest reducer in the in vitro reactions, and its rate of reduction at 1 mM exceeds that of cysteine and glutathione by approximately 13 and 61 times, respectively (Zhitkovich, 2005; Quivryn et al., 2001)."*

which is the exact same sentence that is in the Toxicological Review's Zhitkovich 2005 reference that states on its page 5:

> *"Ascorbate is also the fastest reducer of Cr(VI) in the in vitro reactions, and its rate of reduction at 1 mM concentration exceeds that of cysteine and glutathione approximately 13 and 61 times, respectively (48)."*

Again, this language is a direct quote and needs to be offset in quotation to make that clear.

This type of error also occurs with some frequency in the document and needs to be addressed.

Next, there are numerous instances when the Toxicological Review extensively paraphrases a review article and the meaning of the original passage is altered to another meaning that was not originally intended resulting in some overstatements and inaccuracies. For example, the Toxicological Review states on page 185:

> *"Reduction of hexavalent chromium in vitro produces a large proportion of binary trivalent chromium–DNA adducts, but these have not been detected in vivo. It has been theorized that*

> *the formation of the ternary adducts described above occurs far more frequently due to the high concentration of ligands capable of complexing with trivalent chromium before it can bind to DNA. (Zhitkovich, 2005)."*

Given its general use of "*in vitro*" to mean "in cell culture", the Toxicological Review appears to be stating that binary trivalent chromium-DNA adducts occur in cell culture but not in *in vivo* studies. However, the underlying Zhitkovich review reference actually states that the binary adducts have been detected in a test tube and not in cell culture. Specifically, it states (bold added here for emphasis):

> *"Reductive metabolism of Cr(VI) in vitro usually generates a large number of binary Cr(III)-DNA adducts (22, 37, 53), but the presence of these DNA modifications* ***in cells*** *has not yet been established. The formation of binary Cr-DNA complexes in cells is expected to be strongly inhibited due to the abundance of intracellular ligands capable of rapid coordination to Cr(III) prior to its binding to DNA."*

This error occurs quite often and creates confusion about what the underlying literature is indicating.

There is a reference to "Salnikov and Zhitkovich, 2009" in a couple of places and no citation for this reference is provided. In the citation list, there is one reference listed as "Salnikow, K; Zhitkovich, A. (2008)" and another just below it as "Salnikov, K; Zhitkovich, A. (2008)" that looks to be exactly the same reference. These details should be straightened out and the entire reference section rechecked.

The occurrence of these various errors in citations undermines confidence in the Toxicological Review and raises significant concerns about process. It gives the impression that review articles formed the basis for the evaluation, rather than primary sources and, with the extensive quoting and paraphrasing, that some articles were simply integrated into the document. The use of these review articles in the document needs to be revised and addressed.

C. Confusion due to inconsistent application of the phrases "in vitro" and "in vivo".

The data in the Toxicological Review essentially fall into four groups. There are cell-free system studies, cell culture studies, whole animal studies and human studies. To describe these data, the phrases "*in vitro*" and "*in vivo*" are used. To most readers "*in vivo*" is thought to refer to studies in the body and so include whole animal and in some instances human studies. By contrast, "*in vitro*" is thought to refer to cell culture studies. There are inconsistencies in the use of these terms in the Toxicological Review as some of the underlying references use "*in vivo*" to mean in cell culture and "*in vitro*" to mean in cell free systems. The Toxicological Review has often failed to clarify the underlying studies and carried the underlying language forward into the review.

Two examples of this problem are presented in the preceding criticism, where the Toxicological Review elevated the cells in culture to an "*in vivo*" status, but there are many more occurrences in the document. There are two explanations for this outcome. One possibility is that in some places the Toxicological Review uses "*in vitro*" to mean in cell culture and in others to mean in cell free systems and in some places it uses "*in vivo*" to mean in whole animals and in others to mean in cell culture. The second is that the authors of the Toxicological Review did not realize that the underlying literature meant for "*in vivo*" to mean in cell culture and "*in vitro*" to mean in cell free systems. Regardless of which reason applies, as written the use of the terms is confusing and in some cases, such as the one explained above, misleading. The EPA needs to decide on a definition for these terms, present it, review the underlying literature to be sure they reflect what is meant and then apply them consistently in the document.

D. Failure to check the underlying primary research studies in review articles.

There was a failure to fully consider the underlying primary research articles in the review articles that are extensively cited. This failure creates a perception that the authors did not read beyond that review article, raising questions about process and whether primary data was evaluated at all. Where the document depends on a review article for its source, the underlying primary literature should be checked to confirm the integrity of the statements. One example of this problem is seen in the passage below from page 50 of the Toxicological Review:

> *"An additional important note on these biotransformations regards the interpretation and reliability of data from in vitro assays. In vivo, the intracellular levels of ascorbate are quite high (about 1 mM). In contrast, the levels of ascorbate in tissue culture media are quite low since generally it is not added to the media so that the only source is supplemented fetal bovine serum (FBS). With 10% FBS, the level of ascorbate in tissue cultured cells is only about 50 μM which is 20 times lower than that which is found in vivo (Zhitkovich, 2005). Therefore, experiments on mutagenesis and other toxic effects of hexavalent chromium in tissue culture may underestimate its mutagenic, genotoxic, and cell-transforming activities (Zhitkovich, 2005; Costa and Klein, 2006)."*

Thus, the Toxicological Review wants the reader to question cell culture studies if they do not contain intracellular levels of ascorbate in the mM range. However, the Zhitkovich review article that this section is entirely based upon miscites the primary literature on this matter and it appears the Toxicological Review did not check it. Specifically, as seen in the passage below from the Zhitkovich 2005 review, the claims about physiological levels of ascorbate being in the millimolar range rely on primary research papers that are its references 53 and 54. The Zhitkovich review states:

> *"Under standard tissue culture conditions, A549 and many other human and rodent cells either lack detectable ascorbate or contain it only at micromolar levels (physiological levels are in millimolar range) (53, 54) due to low concentrations of this vitamin in fetal bovine serum and its absence in the most commonly used types of growth media (DMEM, RPMI 1640, F10, F12)."*

These two references are:

> *"(53) Quievryn, G., Messer, J., and Zhitkovich, A. (2002) Carcinogenic chromium(VI) induces cross-linking of vitamin C to DNA in vitro and in human lung A549 cells. Biochemistry 41, 3156-3167.*
>
> *(54) Salnikow, K., Donald, S. P., Bruick, R. K., Zhitkovich, A., Phang, J. M., and Kasprzak, K. S. (2004) Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress. J. Biol. Chem. 279, 40337-40344."*

Examination of these two references shows, however, that neither one offers any data or evidence of physiological ascorbate levels being in the mM range. Salnikow et al. measures the amount of ascorbate loss in cultured cells treated with nickel and cobalt. They do measure ascorbate levels in untreated control cells to determine the background level of their experimental system. But their study does not measure any levels of vitamin C in any physiological setting. Nor does the citation make any reference at all to any study that does.

Quievryn et al., treats the human carcinoma cell line A549 with ascorbate and dihydroascorbate and then measures the amount of vitamin C inside the cell under these experimental conditions. But the study does not measure any levels of vitamin C in any physiological setting. The discussion section does make a comment that: "*Human cells in vivo contain a millimolar concentration of Asc (43)...*". However, that reference 43 is "*43. Meister, A. (1994) J. Biol. Chem. 269, 9397-9400*", which is a review article concerning glutathione and ascorbate in rodents. It contains no mention of ascorbate in human cells.

Thus, the EPA expresses a significant concern about *in vitro* cell culture studies based on a single review article that miscites the primary and secondary literature. This oversight implies that in the preparation of this Toxicological Review, the EPA did not access the primary literature and confirm the secondary and tertiary review articles.

If one were to look at primary literature for ascorbate levels, one would find that these claims are overstated. Ascorbate can reach mM levels in the body, but they are not universally mM levels. For example, Slade et

al., report lung ascorbate levels of 2.91 to 62.35 mg/100 g (Slade, R., Stead, A.G., Graham, J.A., and Hatch, G.E. (1985) Comparison of lung antioxidant levels in humans and laboratory animals. Am. Rev. Respir. Dis. 131(5), 742-6). This measure can be converted to a range of 165 uM to 3.5 mM. However, these are not intracellular levels, but rather the product of tissue homogenization and so a mixture of extracellular and intracellular sources. Thus, there is clearly variability in levels that span the uM and mM range, indicating that this factor may not be so essential.

E. Some exaggerations about Cr transport in cells.

Of less concern, but certainly in need of being addressed is some of the inaccurate language concerning Cr transport into the cell. In several places, cells are described as being impermeable to Cr(III). This characterization is too strong and inaccurate. Cr(III) will enter cells. It is just a slower uptake process than Cr(VI) uptake as Cr(III) moves by simple diffusion and it requires a higher dose to create the concentration gradient to get in the cell. This language should be adjusted.

Also, there is language implying Cr(VI) is actively transported into cells. Cr(VI) does enter rapidly by facilitated diffusion, but it is not an active transport process. These comments should be adjusted.

F. Some typographical errors.

There is also a mention on page 150 that states: 'As discussed in detail in Section 4.4.2 (Intracellular Reduction)..." It is actually section 4.5.2.

In sum, these factors all combine to give the appearance that the mode of action was not fully and consistently considered. To make the document and its conclusions much stronger and more accurate and the rationale behind its decisions more transparent, the following steps should be taken: 1) The EPA needs to separate the genotoxicity literature into discrete endpoints and consider them individually. This consideration should be based on the primary literature, which should be presented in a more careful and coherent fashion so that the reader can understand the strengths, weaknesses and data gaps. 2) Based on that analysis the EPA should choose which lesions are the key lesions and explain the rationale for that choice. 3) Once the key lesions are chosen, the EPA should consider the possible mechanisms of action that may cause those lesions and determine if there are data to support those mechanisms of action. 4) Once the key lesions are identified and the likely mechanisms described, the EPA should explain its rationale for the one chosen to be the mode of action. Of course, non-genotoxic modes of action should also receive similar analysis and presentation. This approach would help the EPA determine the most robust mode of action based on the primary literature. In addition, the EPA should decide what factors are truly confounders of concern and then apply them evenly to all of the literature, reduce its use of secondary and tertiary review articles and improve its management and citation of the literature.

G2. ***Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.***

Although this review is focused on oral exposures, some insight may be gleaned from the inhalation exposure data. Specifically, the data on mutations in lung tumors for Cr(VI)-exposed workers should be considered. These data show a lack of mutations in those tumors suggesting that mutagenicity as considered as a primary change in the DNA sequence is not a key event in the mechanism of action. They are consistent with the fact that one only sees these types of mutations in mammalian experimental models when one forces them by applying very high doses. These studies are:

Katabami M, Dosaka-Akita H, Mishina T, Honma K, Kimura K, Uchida Y, et al. Frequent cyclin D1 expression in chromate induced lung cancers. Hum Pathol 2000; 31 : 973-9.

Kondo K, Hino N, Sasa M, Kamamura Y, Sakiyama S,Tsuyuguchi M, et al. Mutations of the p53 gene in human lung cancer from chromate-exposed workers. Biochem Biophy Res Commun 1997; 239 : 95-100.

Ewis AA, Kondo K, Lee J, Tsuyuguchi M, Hashimoto M, Yokose T, et al. Occupational cancer genetics: Infrequent ras oncogenes point mutation in lung cancer samples from chromate workers. Am J Ind Med 2001; 40 : 92-7.

Hirose T, Kondo K, Takahashi Y, Ishikura H, Fujino H, Tsuyuguchi M, et al. Frequent microsatellite instability in lung cancer from chromate-exposed workers. Mol Carcinog 2002; 33 : 172-80.

Takahashi Y, Kondo K, Hirose T, Nakagawa H, Tsuyuguchi M, Hashimoto M, et al. Microsatellite instability and protein expression of the DNA mismatch repair gene, hMLH1, of lung cancer in chromate-exposed workers. Mol Carinog 2005; 42: 150-8.

Kondo K, Takahashi Y, Hirose Y, Nagao T, Tsuyuguchi M, Hashimoto M, et al. The reduced expression and aberrant methylation of p16INK4a in chromate workers with lung cancer. Lung Cancer 2006; 53 : 295-302.

Ewis AA, Kondo K, Dang F, Nakahori Y, Shinohara Y, Ishikawa M, et al. Surfactant protein B gene variations and susceptibility to lung cancer in chromate workers. Am J Ind Med 2006; 49 : 267-73.

There are also studies of Cr(VI)-induced neoplastic transformation of cells in culture. These need to be considered and included. In particular, reports by Xie et. al., show that cells must acquire a DNA double strand break repair phenotype to undergo transformation indicating escape from repair may be a key event in the mode of action. These studies are:

Patierno SR, Banh D, Landolph JR. Transformation of C3H/10T1/2 mouse embryo cells to focus formation and anchorage independence by insoluble lead chromate but not soluble calcium chromate: relationship to mutagenesis and internalization of lead chromate particles. Cancer Res 1988; 47 : 3815-23.

Xie H, Holmes AL, Wise SS, Huang S, Peng C, Wise Sr JP. Neoplastic transformation of human bronchial cells by lead chromate particles. Am J Respir Cell Mol Biol 2007; 37: 544- 52.

Xie H, Wise SS, Wise Sr. JP. Deficient repair of particulate chromate-induced DNA double strand breaks leads to neoplastic transformation. Mutat Res 2008; 649 : 230-8.

The document only considered mismatch repair, but there are important data showing that other DNA repair pathways must be overcome to induce genotoxicity and carcinogenesis. Double strand breaks and their repair, in particular, are important. The following studies should be added to the repair/DNA double strand break discussion:

Xie, H., Holmes, A.L., Young, J.L., Qin, Q., Joyce, K, Pelsue, S.C., Peng, C., Wise, S.S., Jeevarajan, A., Wallace, W.T., Hammond, D. and Wise, Sr., J.P. Zinc Chromate Induces Chromosome Instability and DNA Double Strand Breaks in Human Lung Cells. Toxicology and Applied Pharmacology, 234: 293–299, 2009.

Xie H, Wise SS, Holmes AL, Xu B, Wakeman T, Pelsue SC, et al. Carcinogenic lead chromate induces DNA double-strand breaks and activates ATM kinase in human lung cells. Mutat Res 2005; 586 : 160-72.

Xie H, Wise SS, Wise Sr. JP. Deficient repair of particulate chromate-induced DNA double strand breaks leads to neoplastic transformation. Mutat Res 2008; 649 : 230-8.

Stackpole MM, Wise SS, Goodale BC, Duzevik EG, Munroe RC, Thompson WD, et al. Homologous recombination protects against particulate chromate-induced genomic instability in Chinese hamster cells. Mutat Res 2007; 625:145-54.

Camrye E, Wise SS, Milligan P, Gordon N, Goodale B, Stackpole M, et al. Ku80 deficiency does not affect particulate chromate-induced chromosome damage and cytotoxicity in Chinese hamster ovary cells. Toxicol Sci 2007; 97: 348-54.

Bryant HE, Ying S, Helleday T. Homologous recombination is involved in repair of chromium-induced DNA damage in mammalian cells. Mutat Res 2006;599:116-23.

Grlickova-Duzevik EG, Wise SS, Munroe RC, Thompson WD, Wise Sr. JP XRCC1 protects cells against particulate chromate-induced chromosome damage and cytotoxicity in Chinese hamster ovary cells. Tox Sci 2006a;92(2):409-15.

Grlickova-Duzevik E, Wise SS, Munroe RC, Thompson WD, Wise Sr JP. XRCC1 protects cells from chromate-induced chromosome damage, but does not affect cytotoxicity. Mutat Res 2006; 610(1-2):31-7.

Vilcheck SK, Ceryak S, O'Brien TJ, Patierno SR. FANCD2 monoubiquitination and activation by hexavalent chromium [Cr(VI)] exposure: Activation is not required for repair of chromium(VI)-induced DSBs. Mutat Res 2006;610:21-30.

Savery LC, Grlickova-Duzevik E, Wise SS, Thompson WD, Hinz JM, Thompson LH, Wise Sr. JP. Role of the Fancg gene in protecting cells from particulate chromate-induced chromosome instability. Mutat Res 2007, 626(1-2):120-127.

There needs to be a stronger and clearer discussion about aneuploidy as a potential mechanism. These studies should be added to that discussion (some are in the document already):
Holmes, A.L., Wise, S.S., Pelsue, S.C., Aboueissa, A., Lingle, W., Salisbury, S., Gallaher, J. and Wise, Sr., J.P. Chronic exposure to zinc chromate induces centrosome amplification and spindle assembly checkpoint bypass in human lung fibroblasts. Chemical Research in Toxicology, 23(2): 386-395, 2010.
Guerci A, Seoane A, Dulout FN. Aneugenic effects of some metal compounds assessed by chromosome counting in MRC-5 human cells. Mutat Res 2000; 469 : 35-40.

Seoane AL, Guerci AM, Dulout FN. Malsegregation as a possible mechanism of aneuploidy induction by metal salts in MRC-5 human cells. Environ Mol Mutagen 2002; 40 : 200-6.

Holmes AL, Wise SS, Sandwick SJ, Lingle WL, Negron VC, Thompson WD, et al. Chronic exposure to lead chromate causes centrosome abnormalities and aneuploidy in human lung cells. Cancer Res 2006; 66: 4041-8.

Wise SS, Holmes AL, Xie H, Thompson WD, Wise Sr JP. Chronic exposure to particulate chromate induces spindle assembly checkpoint bypass in human lung cells. Chem Res Toxicol 2006; 19 : 1492-8.

The following two studies should be added to the clastogenicity results, particularly in light of one reviewer's comments that telomerase may be important as the second paper suggests telomerase does not affect Cr genotoxicity:

Xie, H., Holmes, A.L., Wise, S.S., Gordon, N. and Wise, Sr., J.P. Lead chromate-induced chromosome damage requires extracellular dissolution to liberate chromium ions but does not require particle internalization or intracellular dissolution. Chemical Research in Toxicology, 17(10): 1362-1367, 2004.
Wise SS, Elmore LW, Holt SE, Little JE, Antonucci PG, Bryant BH, et al. Telomerase-mediated lifespan extension of human bronchial cells does not affect hexavalent chromium-induced cytotoxicity or genotoxicity. Mol Cell Biochem 2004; 255: 103-11.

Finally, there are misleading comments about DNA-DNA crosslinks. The Toxicological Review states they are unlikely to form *in vivo*. When one studies the underlying review cited as evidence by Salnikow and Zhitkovich, it becomes apparent that by *in vivo* they mean cells in culture or whole animals. Thus, the review implies that Cr -DNA-DNA crosslinks would not be predicted to occur in cells or whole animals, however, data from Josh Hamilton and Karen Wetterhahn show Cr DNA-DNA crosslinks do form *in vivo*. The inclusion of Josh Hamilton's study showing DNA-DNA crosslinks *in vivo* would correct the inaccurate conclusion in the Toxicological Review that these lesions do not occur *in vivo*. I cannot locate that paper in the time frame available, but Josh Hamilton is a reviewer and should be able to provide it.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Hexavalent Chromium

A1. ***A two-year drinking water study of sodium dichromate dihydrate in rats and mice (NTP, 2008) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.***

This study is the proper study based on the available data. The study is flawed because only very high doses were considered in the study, thus, there is concern that it may not reflect events at lower doses. The EPA is in the unique position that a study that repeats the one above and extends it to lower doses is almost completed. The EPA should wait for the final results of that study to make the most informed analysis.

A2. ***Diffuse epithelial hyperplasia in the duodenum of female mice was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.***

This endpoint is a proper endpoint based on the available data, but is not necessarily a toxic outcome. To allow for better understanding, RfD's for other endpoints should be done and presented including

some continuous endpoints. The EPA may conclude this endpoint is the critical effect, but this approach makes the analysis more transparent, open and clear.

A3. ***Benchmark dose (BMD) modeling was applied to the incidence of diffuse epithelial hyperplasia in the duodenum of female mice to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of diffuse epithelial hyperplasia) scientifically supported and clearly described?***

The BMD modeling has been appropriately conducted and clearly described. To allow for better understanding, BMR modeling at 5% and 1% should be done and presented. This approach makes the analysis more transparent, open and clear.

A4. ***Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.***

The rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD are appropriate. It was suggested that an UF for children and those with different conditions be used. People with different conditions (e.g. antacid use) are already contemplated in the UF applied for interindividual variation. Currently, such a factor for children is not included in the EPA guidelines. It could be that the same interindividual variation may apply in that case as well.

(B) Carcinogenicity of Hexavalent Chromium

B1. ***Under EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgrd.html), hexavalent chromium is likely to be carcinogenic to humans by the oral route of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described?***

The general lack of accuracy in the document in its handling of citing, paraphrasing and considering the underlying literature is of some concern in this presentation. There is a lot of cell culture and animal data showing genotoxicity and clastogenicity, however, the motivation for these studies were largely inhalational exposure-induced cancer. It seems if they can support one route, they could support the other, but it functionally means the data underpinning the oral route of exposure is the one NTP study.

That NTP study is flawed because only very high doses were considered in the study, thus, there is significant concern that it may not reflect events at lower doses. The EPA requirement for a "likely to

be carcinogenic to humans" classification defined as "...appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor "Carcinogenic to Humans." It is understandable why an initial assessment of "likely to be carcinogenic" was chosen as the guidance states: " Supporting data for this descriptor may include: ... an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans".

The next possible descriptor is "suggestive evidence of carcinogenic potential". It is indicated as "...appropriate when the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion." It is also said to cover "...evidence associated with varying levels of concern for carcinogenicity, ranging from a positive cancer result in the only study on an agent...".

Thus, there is conflicting guidance for Cr(VI) on these descriptors. On the one hand, there is a study of multiple species and genders possibly qualifying it for a descriptor of "likely to be carcinogenic to humans". On the other hand there is only one study showing this outcome making it suitable for a descriptor of "suggestive evidence of carcinogenic potential".

In studying the data and descriptors, it appears to be premature to conclude that the weight of the evidence is nearly adequate for demonstrating carcinogenic potential to humans. Moreover, in considering the spirit of the guidelines for the two descriptors, it is clear that "likely to be carcinogenic" descriptor is contemplating that the data concerning multiple species, genders, strains etc. will come from multiple studies not just one. It is also clear that the "suggestive evidence of carcinogenic potential" descriptor is intended for a database with flaws. This database is flawed by the lack of multiple studies and the fact that the one study available relied on very high doses. Accordingly, a descriptor of "suggestive evidence of carcinogenic potential" is more appropriate at this time.

However, the EPA is in the unique position to soon have another study that repeats the NTP study is almost completed. The EPA should wait for the final results of that study to make the most informed analysis. If it too shows tumors at all doses, then the stronger descriptor would be justified.

B2. ***A mutagenic mode of carcinogenic action by all routes of exposure is proposed as the primary mode of action for hexavalent chromium. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for hexavalent chromium that may support an alternative primary mode of action.***

This document is supposed to be limited to an oral drinking water exposure. It is inappropriate to extend any finding to "all routes of exposure" in this document and such evidence has not been considered or presented for dermal or inhalation routes.

In its defense of a mutagenic mode of action, the Toxicological Review states on page 213 that:

> "*In addition to the evidence supporting a mutagenic mode of action in test animals, alternative or additional hypothesized modes of action for hexavalent chromium carcinogenicity have not been demonstrated.*"

There are three concerns with this statement. First, it seems to imply that other modes of action need to be "demonstrated" not simply supported. The frank reality is that no mode of action for Cr(VI) has been demonstrated, even the mutagenic mode of action is only supported and not demonstrated. There should not be a double standard here where the mutagenic mode of action needs to only be supported, while other modes must be demonstrated. The word "demonstrated" should be changed to "supported" to be consistent with the beginning of the passage.

The second concern is that the statement is inaccurate. We supported an alternative mode of action to the induction of mutations in our paper that is cited in the Toxicological Review. Specifically, in Holmes, A.; Wise, SS; Wise, Sr., JP (2008) Carcinogenicity of hexavalent chromium. Indian J Med Res 128:353 – 372, we argue that the mechanism for Cr(VI) does not involve mutations in the primary sequence of the DNA as it is a weak mutagen. Instead, we argue for a genotoxic mechanism leading not to mutations but changes in chromosome number and structure. This point of view is not considered much in the Toxicological Review, but is well-supported in the review and does offer an alternative mode of action that should have been discussed. It is as well-supported as the mutagenic mode of action and so it is inaccurate to state other views have not been demonstrated to the extent a mutagenic mode of action has been.

The third concern with the statement is that it seems to imply that the approach taken in determining the mode of action was to consider those possibilities suggested in reviews of the literature. Therefore, because there is no review article synthesizing the literature to suggest a mode of action, there are no other modes of actions to consider. A better approach would have been to consider the

primary literature and consider some possible modes of action that emerge from the data, but that have not yet emerged as a review article. There are other modes of action that emerge and two possibilities are presented below. There are data to support these modes that should be synthesized, evaluated and considered.

The mutagenic mode of action as the primary mode of action is not sufficiently scientifically supported or described in the Toxicological Review. Many concerns in the presentation with respect to proper citation of results, bias against cell culture studies, and an incomplete consideration of the primary literature are discussed above. The only approach taken was to consider all of these lesions in bulk as simply all representative of mutagenic events and not consider the possible confounding factors for each that may indicate they are not mutagenic events.

A more careful consideration of the primary literature, considering each endpoint on its own merit could argue against a mutagenic mode of action that involves changes to the primary sequence of the DNA strand resulting in mutations. Cr(VI)-induced human tumors rarely contain such mutations and Cr(VI)-induced mutations are most often generated in experimental systems when one artificially forces them to occur by using extraordinarily high doses or systems with compromised repair and cell death pathways or by non-physiological exposure routes. It is unlikely that Cr(VI) is a mutagen at low doses.

The most consistent outcome in the primary literature appears to be impacts on metaphase chromosomes. These outcomes occur at relatively low doses, in intact healthy human cells and across species in cell culture, whole animal and human worker studies. The question remains and this document does not address the underlying mechanism for this outcome. Induction of aneuploidy is another promising mode of action.

One mode of action could involve direct damage to the DNA strand resulting in an alteration in chromosome structure or number. This mode would have key events that include: 1) Uptake of Cr(VI), 2) intracellular reduction of Cr(VI), 3) interaction of reductant products with DNA strands, 4) production of chromosomal changes, 5) escape of DNA repair and apoptosis and 6) expansion of damaged cells.

Alternatively, the mode of action might not involve direct damage to the DNA. Instead, it could involve direct interactions with the mitotic spindle apparatus and be more of an epigenetic event. This mode would have key events that include: 1) Uptake of Cr(VI), 2) intracellular reduction of

Cr(VI), 3) accumulation of intracellular reductant products, 4) interaction of reductant products with mitotic spindle apparatus, perhaps binding to the centrosomes, 5) production of chromosomal changes, 6) bypass of the spindle assembly checkpoint, 7) escape of apoptosis and 8) expansion of damaged cells.

B3. A two-year drinking water study in rats and mice (NTP, 2008) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantification is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

This study is the proper study based on the available data. The study is flawed because only very high doses were considered in the study, thus, there is concern that it may not reflect events at lower doses. The EPA is in the unique position that a study that repeats the one above and extends it to lower doses is almost completed. The EPA should wait for the final results of that study to make the most informed analysis.

B4. The incidence of adenomas and carcinomas combined in the small intestine of male mice from the NTP (2008) two-year drinking water study were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

If one is going to rely on this NTP study, the selection of the incidence of adenomas and carcinomas in the small intestine of male mice from the NTP (2008) two-year drinking water study to serve as the basis for the quantitative cancer assessment is appropriate. However, scientifically these lesions are not the same and are not necessarily linked. Thus, one or the other should be used.

B5. The oral slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk of tumors of the small intestine in male mice). Has the modeling been appropriately conducted and clearly described?

The modeling has been appropriately conducted and clearly described. More methods are needed to make it clearer. It is possible that Cr(VI) acts via a threshold. The EPA is in the unique position that a study that repeats the NTP study and extends it to lower doses is almost completed. This study will help clarify if there is a threshold. The EPA should wait for the final results of that study to make the most informed analysis.

POST-MEETING COMMENTS SUBMITTED BY

Anatoly Zhitkovich, Ph.D.

Review of a draft of *Toxicological Review of Hexavalent Chromium* prepared by the US-EPA in Support of Summary Information on the Integrated Risk Information System (IRIS)

By Anatoly Zhitkovich, Ph.D.
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General Charge Questions:

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazard?

In general, I found the Draft to be well prepared and balanced in its presentation of various aspects of chromium-6 toxicology and carcinogenesis. It has a logical structure, leading a reader from the basics of redox chemistry of chromium-3 and chromium-6 and their interactions with biosystems to the detailed description of *in vivo* studies on bioavailability, tissue disposition and finally, toxic and carcinogenic effects. Weaknesses and strengths of the key *in vivo* studies along with the reasons for the inclusion or exclusion of specific findings were also clearly presented. Different sections vary somewhat in their degree of emphasis on the importance of one or another mechanistic aspect of Cr(VI) toxicology, which is also reflective of divergent opinions in the field. As typical for any large document covering a complex topic, the Draft contains some information that is not up-to-date and would benefit from additional editorial work.

Suggested modifications and corrections:

1) Section 2.1 "*Environmental Sources and Occurrence*" appears to draw a large amount of information from decades-old literature. The analytical approaches for the detection of both total Cr and Cr-6 underwent major improvements in 1980s and the older references to the amount of Cr-6 in various environmental media and biological samples should be looked at with a healthy degree of skepticism and scrutinized for potential overestimations. My specific concerns are related to the included values for Cr-6 levels in soil, freshwater and seawater. All three sets of values are too high for the typical samples from the noncontaminated areas/sites.

2) Table 2-1 "*Industrial uses of hexavalent chromium compounds*" is missing uses of sodium/potassium chromate and dichromate. The addition of information on sodium dichromate is particularly important in light of its testing for carcinogenicity by the NTP.

3) p.14, last para: Cr(III) oxidation to Cr(VI) by atmospheric oxygen can also occur in the presence of calcium oxide (Pillay et al. 2003).

4) Table 2-4 "*Detection limits for methods…*" reports outdated values. The EPA's Method 218.6 for Cr(VI) in water has a detection limit which is ~100 times lower than detection limits listed in Table 2-4. A recent modification of this method affords detection of Cr(VI) at the 0.003 ppb level (Application Update 179 from Dionex). The detection sensitivity of flame AAS is also underestimated. Based on the discussion of work by Levine (2007) on p.16 and other available literature, the detection limit for total Cr in water samples by ICP-MS reported in Table 2-4 is probably lower by a couple orders of magnitude.

5) p.25, lines 5-6: in Donaldson and Barreras (1966), urinary excretion for orally administered Cr(VI) and Cr(III) were 2.1 and 0.5%, respectively (not 2.1 versus 1.5%).

6) Table 3-5 and the discussion of these results appear contradictory.

7) Table on p.39 is confusing: it reports daily doses of sodium dichromate dihydrate in the NTP-2008 study but the ratio mice:rats looks incorrect based on the data in the top two rows. It is also unclear why Cr(VI) consumption was compared between male rats and female mice and not between animals of the same sex.

8) Finley et al. (1996) delivered a Cr-6 dose of 0.005 mg/kg/day, not 0.005 mg (p.45).

9) Section 3.3 describes Cr(VI) reduction by microsomal enzymes in detail on three pages. This degree of attention may create an erroneous impression about the importance of the specialized enzymatic processes in Cr-6 metabolism. There is a strong consensus in the field that Cr(VI) reduction in mammalian cells is primarily accomplished nonenzymatically by ascorbate and small thiols such as glutathione and cysteine. As acknowledged in other sections of the draft, ascorbate alone accounts for reduction of 80-95% Cr(VI) depending on the tissue (Standeven and Wetterhahn, 1991,1992). A combined contribution of ascorbate and thiols is responsible for more than 95% Cr(VI) reduction. These estimates from tissue preparations were confirmed by the measurements of individual reduction rates (Quievryn et al. 2003). It is clearly important to present mechanistic aspects of Cr(VI) reduction but the detailed focus should be on ascorbate and non-protein thiols, not enzymatic systems with a minimal contribution to the overall Cr(VI) metabolism *in vivo*. The absence of Cr(V) intermediate during Cr(VI) reduction by ascorbate is especially important.

10) Last sentence on p. 50: The description of vitamin C accumulation by cells is not entirely correct. Cellular accumulation of vitamin C via uptake of its oxidized form dehydroascorbic acid is a *physiological* mechanism that functions in all mammalian cells. It is particularly active in human cells, which leads to very efficient recycling and much lower daily requirements for vitamin C in humans compared to rodents (Nualart et al. 2003, Montel-Hagen et al. 2008). These differences between humans and rodents are relevant for the interspecies extrapolation.

11) Figure 3-6 needs to be modified:

    a) Depiction of the cation channel with the comment "No effect" could be interpreted as indicating some nontoxic delivery route for chromium. Unlike some other toxic metals, cation channels play no role in uptake of Cr ions and the cation route should be deleted from this Figure.

    b) Although some Cr(III)-ligand complexes can exhibit a limited ability to enter cells, there is no evidence that they can react with DNA and cause mutagenic/genotoxic ternary Cr-DNA adducts, as shown in the Figure. The Figure should be modified by removing this nonexistent route of DNA damage.

    c) The route for the formation of DSB by mismatch repair needs to be revised. As demonstrated in a recent study by Reynolds MF et al (2009), ternary Cr-DNA adducts are directly bound by mismatch repair proteins followed by DSB formation in G2 phase without stalling replication forks in the preceding S-phase.

12) Summary Table 4-20 should add the *+(M) designation* for mutagenesis of sodium dichromate in laboratory animal, as demonstrated by Cheng et al. 2000 (this study was later cited in Table 4-23).

13) Table 4-23 "In vivo genotoxicity studies... Mutations section" is missing references to two positive mutagenesis studies in vivo by Itoh and Shimada (1997, 1998).

14) Section 4.7.3.1. *Hypothesized Mode of Action*:

a) Ref. to Salnikow et al. (1992) in support of ternary complexes is inappropriate and could be replaced by Voitkun et al. (1998).

b) Neither Zhitkovich (2005) nor Voitkun et al. (1998) dealt with "intrastrand DNA-DNA crosslinks". A study by Lloyd et al. (1998) is the only original report describing putative intrastrand crosslinks, which were generated in a buffer solution with massive concentrations of hydrogen peroxide. Tested under the same reaction conditions, essential metals copper and cobalt were even more potent inducers of these presumed crosslinks. There is no evidence for the formation of these crosslinks by chromium-6 in cells or in acellular systems containing its main biological reducers.

15) Tables 4-22 and 4-23 failed to include any references to studies reporting the formation of chromium-DNA adducts in cultured mammalian cells and *in vivo*. This is a critical omission as the presence of chromium-DNA adducts demonstrates a direct DNA-damaging mechanism for Cr(VI) genotoxicity. The formation of DNA adducts was briefly discussed in other sections of the Draft.

16) Discussion of a negative report on DNA damage by DeFlora et al. (2008) on pp.206-207:

This study found no evidence of DNA damage in forestomach, glandular stomach and duodenum of female SKH-1 mice after a 9-month long exposure to 5 and 20 mg/L Cr(VI) in drinking water. Based on the study by DeFlora et al. (2008), a high safety threshold argument was also made in some of the submitted public comments. The Draft argued that a shorter duration of exposure (9 months vs. 2 years in the NTP study) made the DeFlora 2008 study "infeasible" for the comparison. With the exception of mutations and a potential accumulation of unrepaired damage in a population of the long-lived crypt stem cells, there are no other obvious factors suggesting that the formation of DNA damage by Cr(VI) in the entire duodenum during the first half of the 2-year exposure would be significantly different from damage occurring at the end of the 2 years.

However, the study by DeFlora et al. (1998) is uninformative for other reasons. The authors assayed tissues for two forms of DNA damage: DNA-protein crosslinks and 8-oxo-dG (the Draft incorrectly described 8-oxo-dG as a DNA adduct; it is actually a base oxidation product). Both types of damage showed no increases above background in tissues of exposed animals; however, these negative results were predictable based on the technical limitations of their analytical methodologies. Since Cr(VI) tumorigenesis occurred in the duodenum, I will limit my discussion to this tissue.

1) DNA-protein crosslinks:

A positive control consisting of mouse duodenal cells treated *ex vivo* with 1.6 mM Cr(VI) (83.2 mg/L) generated a 2.5-fold response. Such a low responsiveness was clearly insufficient to detect DNA damage for exposures with 4.2- and 16.6-times lower Cr(VI) levels in the 20 mg/L and 5 mg/L test groups, respectively, even in the unlikely scenario of no reduction and no dilution of Cr(VI) with stomach juices before reaching the duodenum. Although chronic exposures frequently leads to the accumulation of unrepairable damage, a dramatic increase in DNA-protein crosslinks during chronic Cr(VI) exposures would be not very likely. DNA-protein crosslinks are repairable lesions in mammalian cells *in vivo* and culture (Tsapakos et al. 1983, Sugiyama et al. 1986, Zecevic et al. 2010) with the possible exception of human peripheral blood lymphocytes (Quievryn and Zhitkovich 2000). Furthermore, ongoing proliferation and shedding of cells in the duodenal villi would result in continuous dilution of damage and loss of previously exposed cells.

2) 8-oxo-dG measurements:

A positive control generated by exposure of mouse duodenal cells to 1.6 mM Cr(VI) (83.2 mg/L) produced a 3.8-fold increase in the levels of 8-oxo-dG. It is doubtful that this assay sensitivity was sufficient to detect significant increases in 8-oxo-dG levels for even undiluted/unreduced 20mg/L and 5mg/L Cr(VI) concentrations that were used in the treatment groups. 8-oxo-dG as a biomarker of DNA damage has one critical limitation – a short lifetime due to its rapid removal by base excision repair. Repair of 50% 8-oxo-dG occur within 30 min and is complete within 2 hr (Lan et al. 2004). Not only would this short lifetime prevent any accumulation of 8-oxo-dG during chronic exposures, but it would also make it very difficult to detect this lesion even after recently ingested water with a sufficiently high dose producing positive responses under *ex vivo* conditions.

G2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

The Draft included information from all major studies that have a significant impact on the main conclusions. It does not list or discuss every study published on Cr(VI) but there was also no systematic exclusion. In Section 4.4, I would recommend adding an important report by Gibb et al. (2000), which is the largest epidemiological study of cancer risk due to inhalation exposure to Cr(VI). While the omission or inclusion of this study does not change the overall conclusion about Cr(VI) carcinogenicity to humans via inhalation, Gibb et al. (2000) provided strong evidence of chromate dose-dependence for lung cancer risk and its independence of the common confounder, tobacco smoking.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Hexavalent Chromium

A1. A two-year drinking water study of sodium dichromate in rats and mice (NTP, 2008) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

The NTP-2008 is the best available study of chromium-6 toxicity via oral exposure and its choice for the calculation of the RfD is scientifically sound and was clearly explained in the Draft.

A2. Diffuse epithelial hyperplasia in the duodenum of female mice was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

The incidence of diffuse epithelial hyperplasia in the duodenum of female mice was the most sensitive histological response observed in Cr(VI)-exposed groups and therefore, it was appropriately selected as the critical effect for the RfD.

A3. Benchmark dose (BMD) modeling was applied to the incidence of diffuse epithelial hyperplasia in the duodenum of female mice to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described. Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of diffuse epithelial hyperplasia) scientifically supported and clearly described?

BMD modeling and the calculations of the POD both appear to be appropriately performed.

Anatoly Zhitkovich, Ph.D.

A4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described? If changes to the selected UFs are proposed, please identify and provide the rationale.

Two UFs were applied: UF=10 for interspecies extrapolation to humans and UF=10 for interindividual variability in the human population. The interspecies UF was used because there is no available information to quantitatively assess the true differences in chromium-6 toxicokinetics and toxicodynamics between humans and laboratory rodents. There are, however, two biological factors that point to a potentially greater sensitivity of humans relative to mice. The first is related to the fact that telomerase was shown to suppress genetic damage by chromium-6 (Glaviano et al. 2006). All mouse cells express telomerase while only stem cells retain telomerase expression in human tissues. The second factor is the difference in ascorbate metabolism. Human cells actively recycle ascorbate (Nualart et al. 2003, Montel-Hagen et al. 2008), resulting in ~100 times lower requirements for this vitamin by humans relative to rodents. A more economical use of vitamin C by humans also results in lower ascorbate concentrations in the extracellular fluid (for example, as reported for bronchoalveolar lavage fluid by Slade et al. 1993), which would more rapidly detoxify chromium-6 via extracellular reduction. However, No specific information about ascorbate concentrations in the extracellular environment of the duodenum and jejunum of mice and humans is currently available.

The interindividual variability in sensitivity to chromium-6 was not studied and the application of the safety coefficient (UF) is definitely appropriate in this case. However, the proposed UF=10 likely underestimates the range of the interindividual variability. The Draft has a brief discussion on the common presence of genetic polymorphism in DNA repair genes as one source of interindividual differences. Four major DNA repair pathways (mismatch repair, nucleotide excision repair, base excision repair and homologous recombination) are known to impact the extent of genetic damage and cytotoxicity by Cr(VI), and the use of UF=10 to account for interindividual differences in the overall DNA repair would assume a quite low degree of variability for each repair process (overall 10-fold variation would result from a very narrow 1.8-fold variation in each process: $1.8^4 = 10.5$).

Chromium-6 toxicity can be affected on three levels: 1) differences in extracellular detoxification, 2) differences in cellular uptake and 3) differences in cellular/genomic defense mechanisms. A 5-fold variation at each stage would give a potential 125-fold variation in the general population. A study by Donaldson and Barreras (1966) showed that individuals with pernicious anemia had 4-times higher systemic uptake of chromium-6 due to its lower detoxification in the stomach. Widespread use of antacid medications has a clear potential to diminish reduction rates of chromium-6. No systematic studies on potential variations in chromium-6 uptake have been performed yet, but two human lung carcinoma lines, H460 and A549, displayed a 5-fold difference in chromium-6 accumulation (Macfie et al. 2010). A caveat of using information from these two cell lines is that they are malignant and therefore it is not possible to determine whether their differences were present in the initial normal cells or whether it is a side effect of different transformation processes.

The Draft correctly stated on p.214 that there is no information about susceptibility of children to chromium-6 toxicity. In this case, it would be clearly appropriate to use additional UF=10 to account for a potential early life susceptibility. If EPA considers it unnecessary, then the exclusion of this UF should be justified in Section 5.1.3. My recommendation would be to use UF=100 to account for the combined effects of the interindividual variability in susceptibility and early life exposures.

Anatoly Zhitkovich, Ph.D.

(B) Carcinogenicity of Hexavalent Chromium

B1. Under EPA's 2005 Guidelines for Carcinogen Risk Assessment, hexavalent chromium is likely to be carcinogenic to humans by the oral route of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described.

The classification of hexavalent chromium as "likely to be carcinogenic to humans" via the oral route of exposure is supported by evidence of its tumorigenicity in the oral cavity of female and male rats and in the small intestine of female and male mice. An increased incidence of stomach cancers in the JinZhou area (China), which was contaminated with high concentrations of chromium-6 in drinking water, is supportive of the selected classification. Even if the ecological study from China is excluded, the weight of evidence from animal studies is adequate to designate hexavalent chromium as "likely to be carcinogenic to humans" via the oral exposure.

B2. A mutagenic mode of carcinogenic action by all routes of exposure is proposed as the primary mode of action for hexavalent chromium. Please comment whether this determination is scientifically supported and clearly described. Please comment on data available for hexavalent chromium that may support an alternative primary mode of action.

Mutagenic mode of action: Hexavalent chromium was overwhelmingly positive for genotoxicity in a large variety of cells and organisms. It was also consistently mutagenic in bacterial and mammalian test systems. The mutagenicity and genotoxicity of Cr-6 result from a direct DNA-damaging mechanism, as evidenced by the induction of mutagenic chromium-DNA adducts and other forms of DNA damage. Formation of chromium-specific DNA lesions at environmentally relevant Cr-6 concentrations and sensitivity of genotoxic responses to manipulations of cellular DNA repair further support the role of direct DNA damage as a primary cause of genotoxicity. Since Cr-6 is taken up via ubiquitously expressed sulfate transporters and its metabolism in cells occur via ubiquitously present ascorbate, glutathione and cysteine, there is no reason to believe that the formation of DNA damage in the small intestinal cells and in more extensively studied cell types would be significantly different. Thus, diverse lines of evidence are fully consistent with a mutagenic mode of carcinogenic action for hexavalent chromium. The Draft clearly presented the main arguments for this designation. However, as pointed out above, Tables 4-22 and 4-23 need to be supplemented with information on Cr-DNA adducts.

Supporting the importance of Cr-DNA adducts in chromate tumorigenicity are findings from the MOA study by the ACC in which levels of adducts were dramatically higher in the duodenum and jejunum of mice vs. rats. This result mirrors species differences in the intestinal carcinogenesis by chromate and it could not be explained by differences in tissue accumulation of chromium.

In contrast to clear positive mutagenicity and genotoxicity data from *in vivo* studies and ascorbate-restored mammalian cell cultures, aneuploidy and epigenetic responses have not yet been tested in animal models and so far have been observed only in ascorbate-deficient cells. In fact, Sun et el. (2009) have found that the induction of epigenetic changes by chromate in human cultured cells occurs only under ascorbate-depleted conditions.

The measurements of mutations in KRAS and p53 genes as part of the MOA study sponsored by the American Chemistry Council would not necessarily provide a clear answer about the mutagenic mode of action. A short 3-months duration of this study vs. 2 years for the NTP bioassay certainly diminishes its ability to detect mutations. Among the proposed mutation readouts, three KRAS codons represent a very small and consequently, insensitive mutagenic target. This gene was only rarely mutated in chromate-associated human lung cancers (Ewis et al. 2001). The p53 gene is also uncommonly mutated in cancers

among chromate workers (Kondo et al. 1997). The presence or absence of KRAS or p53 mutations do not serve as a strong test for the validity of the mutagenic mode of carcinogenic action, as the frequency of cells with mutated KRAS or p53 can increase through selection of the pre-existing mutant clones whereas transformation process can result from mutagenic events in other components of KRAS and p53 pathways. For example, a large wave of early thyroid cancers among Chernobyl radioiodine-exposed children was caused by translocations in growth factor receptors with almost no RAS and p53 mutations (Nikiforov et al. 1996, Suchy et al. 1998, Williams 2002).

Potential alternative modes of action: Two lines of in vivo evidence have been presented to point to a potentially nonmutagenic mode of carcinogenic action. One is based on the drinking water study by DeFlora et al. 2008, which found negative results for DNA damage in the duodenum of mice. However, as discussed in detail above, this study used assays that were insensitive for detection of DNA damage by the employed doses of Cr(VI)in drinking water. Therefore, the negative results of this work were expected and therefore, uninformative.

The other observation leading to the discussion of nonmutagenic or indirectly mutagenic mechanisms of carcinogenicity was the presence of diffuse epithelial hyperplasia in the NTP bioassay. Although the NTP study has not found significant necrosis in the small intestine of exposed mice, it is quite possible that the observed hyperplasia was a typical manifestation of regenerative responses. A combination of increased proliferation and inflammation could be presented as an alternative mechanism for indirect induction of mutations due to higher rates of cell division and by reactive oxygen species released by the recruited inflammatory cells. This carcinogenic pathway would exhibit a strongly sublinear, threshold-type dose dependence, as it relies on the induction of cell death and small doses would not kill cells. Inflammatory events could also be linked to cell death of chromium-damaged cells, which release pro-inflammatory molecules. The extent of hyperproliferation in chromium-exposed groups was modest, and considering the overall very high rate of cell division in the small intestine, it is hard to see how somewhat faster replication would provide dramatically more spontaneous mutations required for cancer development. At best, the cytotoxicity-induced compensatory proliferation mechanism and the mutagenic mode should co-exist at high tumorigenic doses.

The results from the MOA study sponsored by the ACC argue against significant inflammatory responses in the duodenum of chromate-exposed mice, as no increases in the levels of 8-oxodG and in the panel of 22 cytokines have been observed. A statistically significant drop in the ratio of GSH/GSSG was small in its magnitude, further demonstrating that tumorigenic doses were not associated with the state of strongly elevated oxidative stress and inflammation.

The presence of chromium-induced hyperplasia could also be viewed as a manifestation of cancer-protective responses by the small intestine. Elimination of genetically damaged cells by apoptosis or another form of cell death is a firmly established protective mechanism against cancer. Thus, there are two opposing interpretations for the toxicological significance of the observed hyperplasia: one is pro-tumorigenic and another is anti-tumorigenic. The supralinear shape of dose-tumor incidence responses in the NTP-2008 studies for female mice is consistent with the engagement of cancer-protective mechanisms. Tumor incidence vs. dose in male mice visually displayed a linear dose-dependence (as shown in Stern 2010). Thus, a hypothetical cytotoxicity-based mechanism with the expected dose-response sublinearity is contradicted by the available evidence.

B3. A two-year drinking water study in rats and mice (NTP, 2008) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantitation is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

The selection of a two-year drinking water study in rats and mice by the NTP (2008) for the calculation of an oral slope factor is appropriate. The NTP study was well designed and well executed. No other multiple-dose chronic oral carcinogenicity study in animals is available and the dose-dependence from the single ecological study linking chromium-6 in drinking water to human stomach cancers cannot be reliably estimated.

B4. The incidence of adenomas and carcinomas combined in the small intestine of male mice from the NTP (2008) two-year drinking water study were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether the selection is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

The choice of the combined incidence of adenomas and carcinomas in the small intestine of male mice from the NTP-2008 study for the quantitative cancer assessment was based on a better fit of the multistage model for the male mouse data than for the female mouse data. However, it was unclear why a combination of male and female mouse data sets was not used.

B5. The oral slope factor was calculated by linear extrapolation from the POD. Has the modeling been appropriately conducted and clearly described.

The calculation of the oral slope factor from the POD was appropriately performed. As per US-EPA 2005 Guidelines for Carcinogen Risk Assessment, a linear extrapolation to low doses was used based on the selection of the mutagenic mode of carcinogenic action for chromium-6.

The ability of ingested chromium-6 to cause adverse effects at both environmentally relevant and much higher doses has been questioned, given the reported high chromate reducing capacity of the gastric juice and a limited systemic penetration of chromium after oral exposure (as extensively reviewed by the Draft). These considerations led to the formulation of the threshold model of chromium-6 carcinogenesis, which postulates that only doses that exceed the reducing capacity of the tissue (stomach for ingestion exposures) would be carcinogenic (DeFlora 2000). This model would argue that despite the selection of a mutagenic mode of action with the resulting recommendation for default linear extrapolation, the complete detoxification of low-to-moderate chromium-6 doses in the stomach makes it inappropriate to perform linear extrapolation from the POD.

The ability of gastric juices to reduce/detoxify chromium-6 is generally accepted in the field; however, studies with human volunteers and other considerations argue against the completeness of the detoxification process. For example, the bioavailability for Cr(VI) was ~10-times higher than for Cr(VI) reduced with orange juice prior to ingestion (Kuykendall et al. 1996). The extent of chromium-6 reduction in the stomach is influenced by three factors: its reduction capacity, reduction rate and stomach emptying time. Based on the reported high reduction capacity of the stomach (>80 mg/day, DeFlora et al. 1997), the rate of reduction by gastric juice under fasting conditions could exhibit pseudo-first order kinetics for a broad range of chromium-6 concentrations. This means that the percentage of reduced chromium-6 could be the same for both very small amounts and much larger amounts. Reduction of chromium-6 by artificial gastric juice has been found to follow first order reaction kinetics (Gammelgaard et al. 1999). Consistent with the first-order reaction kinetics, Donaldson and Barreras (1966) found that human subjects excreted in the 24-hr urine 2.1% of ingested 20 ng radioactive chromium-6 whereas Kerger et al. (1997) found that ingestion of 5 mg chromium-

6 by human volunteers led to about 1.43% excretion during the first 24 hr (1.43% excretion is a conservative 1/4th estimate from the average 4-day excretion value of 5.7%). Thus, the bioavailability of 20 ng and 5 mg chromium-6 (250,000-fold range) looks quite similar.

Donaldson and Barreras (1966) also performed a very important experiment on the bioavailability of 20 ng radioactive chromium-6 that was directly delivered into the duodenum of human subjects. In this case, they found that 10.6% of chromium was excreted in the urine. Since the duodenal delivery represent 100% nonreduced chromium-6, then the amount of nonreduced chromium-6 in their oral route experiment can be estimated from the urinary excretion of 2.1% divided by 0.106 = 19.8%. For the study by Kerger et al (2007), the same type of calculations gives an estimate of 14.3% nonreduced chromium-6 reaching the duodenum. Gammelgaard et al. (1999) calculated a half-life of 23 min for reduction of 0.1mg/L chromium-6 (current MCL for total chromium) by artificial gastric juice. After 1 hr, this reduction rate would leave 16.5% chromium-6.

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POST-MEETING COMMENTS SUBMITTED BY

Yiliang Zhu, Ph.D.

Post Meeting Review Comments on EPA's

Toxicological Review of Hexavalent Chromium

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G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazard?

This EPA's Review is well organized overall and for most part well presented. The literature review is extensive and thorough. However, the Review does not contain a set of clearly-stated criteria under which the literature was searched, critiqued, and synthesized. Specifically, was each published study judged with respect to design (including sample size), exposure assessment, choice of dose metrics, choice of endpoints, adequate dose-response data, dose-response modeling, and positive findings? Whereas some of these criteria may have been used in the Review, the lack of a systematic approach may have compromised the consistency and transparency of this review process. In its independent review of the EPA's IRIS Documents on Formaldehyde and Dioxins, for example, the National Academies of Science and National Research Council have strongly advocated the adoption of a systematic review approach to EPA's IRIS risk assessment process (NAS 2006, 2011). The present Review of Hexavalent Chromium once again demonstrates the need for adopting a systematic review approach.

G2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

NA

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Hexavalent Chromium

A1. A two-year drinking water study of sodium dichromate dihydrate in rats and mice (NTP, 2008) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

The Review offers EPA's rational for selecting the NTP's two-year drinking water study of sodium dichromate dihydrate in rats and mice (NTP, 2008). EPA's justification includes the lack of reliable epidemiological data, solid design of the NTP's experiment, its controlled exposure regimens, the sensitivity of the endpoint, the availability of dose-response data, and consistency with hypothesized

genotoxicity MOA. These justifications are acceptable. There are still merits to include other studies, particularly the 3-month sodium dichromate dehydrate drinking water exposure of rats and mice (NTP 2007) in calculating RfDs. Inclusion of additional and all qualified studies is especially beneficial for better quantifying uncertainties and variations arising from different studies due to different study designs, strain/species of animals, and exposure regimens. As in a systematic review, studies meeting selection criteria should all be included for review and for analysis. Selecting a final RfD then becomes a risk management decision.

A2. Diffuse epithelial hyperplasia in the duodenum of female mice was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

EPA considered seven non-cancer endpoints for deriving RfDs (Table 5-1): chronic liver inflammation in female rats, histiocytic cellular infiltration in the liver of female mice, diffuse epithelial hyperplasia in the duodenum of the male and female mice, histiocytic cellular infiltration in the mesenteric lymph nodes of male and female mice, and cytoplasmic cellular alteration of acinar epithelial cells in the pancreas of female mice. All seven are quantal response from the NTP's 2-year chronic exposure study. The selections were largely driven by the dose-response data these effects exhibited. After dose-response modeling and the estimation of benchmark dose (BMD) for each of these select effects, diffuse epithelial hyperplasia in the duodenum of the female mice was chosen as the critical endpoint simply because it yielded the smallest BMD and its corresponding lower confidence limit (BMDL). It must be noted that the dose-response model for this critical effect was done only after deleting the two highest doses. As a result, the dose-response modeling relied on only three dose level (including the control), leaving little room for any flexible dose-response forms other than the "linear" multi-stage model with a polynomial of 1 degree of freedom.

Instead of relying on a select "critical" effect, EPA could report a range of RfDs based on a set of qualified and select effects. As a result, EPA will be able to a range of RfDs, projecting the uncertainty and variation of RfDs arising from man y sources and affording risk management the opportunity to make an informed choice of a final RfD (NAS, 2010). This is important as EPA is moving towards enhancing analysis of uncertainty and variation in risk assessment.

To this end, EPA could have benefited greatly by including additional endpoints from this principle study as well as other qualified studies. Potential candidates include histiocytic cellular infiltration in the duodenum of female rats and male mice, histiocytic cellular inflammation in pancreatic lymph nodes of male rats, and histiocytic cellular infiltration in the liver of female rats. EPS considered only the quantal responses in this Review for the purpose of computing RfDs. It is unclear why effects of continuous measurement scale were not considered. Many of these effects show

unequivocal dose-response (e.g. Tables 4-12 and 4-13) and seem to be relevant to the hypothesized MOA of hexavalent chromium. The availability of EPA's software BMDS for dose-response modeling and benchmark dose computation makes it practical and useful to consider continuous effects for RfD derivation as well.

A3. Benchmark dose (BMD) modeling was applied to the incidence of diffuse epithelial hyperplasia in the duodenum of female mice to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of diffuse epithelial hyperplasia) scientifically supported and clearly described?

EPA should be commended for conducting BMD modeling for multiple effects with different model forms. For the modeling of the incidence of diffuse epithelial hyperplasia in the duodenum of female mice, EPA should provide a more detailed discussion on the limitation of the dose-response modeling (See A2). Uncertainties due to model choice, variation in the shape of seemingly equally well-fit models also can be quantified to a degree by considering multiple benchmark response levels (BMR) for each model. EPA used only BMR=10%. It makes perfect sense to also consider BMR=5% or even BMR=1% when such a choice is supported by the data. This is the general recommendation of EPA's own guideline (EPA, 2000). By doing so EPA would be able to quantitatively demonstrate uncertainty and variations due to the choice of different models and different BMR levels. Additionally, EPA should briefly but clearly define the BMD concept and methodology in an appendix to improve the readability for readers unfamiliar with the process.

A4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

The use of uncertainty factors (UFs) in this review is well described and is consistent with EPA's guidance documents for RfDs. Exposure in earlier stage of life was discussed.

(B) Carcinogenicity of Hexavalent Chromium

B1. Under EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgrd.html), hexavalent chromium is *likely to be carcinogenic to humans* by the oral route of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described?

The descriptor of "likely to be carcinogenic to humans" for hexavalent chromium is consistent with EPA's Guidelines for Carcinogen Risk Assessment. EPA gave a clear description of the hypothesized MOA.

B2. A mutagenic mode of carcinogenic action by all routes of exposure is proposed as the primary mode of action for hexavalent chromium. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for hexavalent chromium that may support an alternative primary mode of action.

A mutagenic mode of action was proposed as the primary mode of action. On the one hand, EPA discussed data gap and uncertainties about the mutagenic MOA and other possible MOAs. On the other hand, EPA defended the mutagenic MOA despite the lack of data evidence. For example, the only animal study that investigated target tissue genotoxicity (De Flora et al. 2008) reported negative results for DNA-protein crosslinks and DNA adducts in forestomach, glandular stomach, and duodenum of mice exposed to hexavalent chromium in drinking water for 9 month. EPA dismisses the negative finding on the basis of a shorter duration of the study compared with the 2-year NTP study.

B3. A two-year drinking water study in rats and mice (NTP, 2008) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantification is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

The selection of the NTP's 2-year drinking water study in rats and mice (NTP, 2008) is justified. Reasons for why existing epidemiological data were not used for estimating cancer slope factor are acceptable.

B4. The incidence of adenomas and carcinomas combined in the small intestine of male mice from the NTP (2008) two-year drinking water study were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

For dose-response assessment EPA considered the incidences of adenoma and carcinoma combined in the small intestine of male and female B6C3F mice (Tables 5.3 and 5.4), in the oral cavity (mucosa and tongue) (Tables 5.3 and 5.4) in rats (NTP 2008). (Note the denominators that determine the tumor incidence in small intestine are not consistent with those in Table 4.19). EPA did not consider the incidence of other neoplasm because the incidence is not dose-dependent.

The incidence of adenoma or carcinoma in the oral cavity in both male and female rats elevated only at the highest dose, but not at the three lower doses (up to 2.1 and 2.4 mg/kg-d for male and female respectively). To fit a dose-response model to these incidence data that exhibited hockey-sticker shape of dose-response requires a nonlinear (curve-linear) functional form or even a threshold model. Such curve-linear pattern seems inconsistent with the hypothesized genotoxic MOA. The lack of dose-response in these two endpoints was cited as reason for not advancing these two endpoints for final dose-response analysis. Better justification is needed.

B5. The oral slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk of tumors of the small intestine in male mice). Has the modeling been appropriately conducted and clearly described?

EPA carried out dose-response modeling and BMD estimation for the incidences of adenoma and carcinoma of small intestine in male and female mice separately. EPA stated that it relied on the multi-stage model because the model is preferred by the agency, but gave no justification or explanation. It went on to report an estimated slope of 0.09 (mg/kg-day) and 0.10 (mg/kg-day) for male and female mice respectively. In section 5.3.4 of the Review, EPA reported the CSFs derived on the basis of the cancer incidence of small intestine in male and female mice, and chooses that of male mice because of "the poor fit of the multistage model to the female mouse data". EPA did not provided adequate detail on the modeling efforts, or a discussion and justifications for its final selection (section 5.3.3 and 5.3.4). EPA did provide some detail in Appendix B2, which is essentially the direct output from running the BMDS software, but again no discussion or explanation of the output. It would be helpful and necessary that EPA substantially expand sections 5.3.3 and 5.3.4 to report in greater detail the modeling process, the issues encountered, and justify the decision and choice therein.

On a more technical side, examination of Appendix B2 reveals that (1) no standard error is reported for the estimate of model coefficient, and (2) the coefficient of the second order in the polynomial was set to be zero, not estimated. The model did not fit the data at all despite a non-significant p-value for goodness-of-fit test. EPA did not give any explanation or discussion in this regard. The significance of a goodness-fit-test depends on sample size, and a non-significant result does not imply a correct model, especially within a range where there are no data. EPA should explore different options: trying different models, considering omitting the highest dose, or considering combine male and female mice.

Inclusion of multiple studies, multiple endpoints, multiple model choices, and various BMR levels for deriving a POD is increasingly desirable towards a more systematic and quantitative risk assessment paradigm. It will afford an opportunity to quantify the underlying uncertainties and variations associated with the choices and options made each many stages of the risk assessment process. Within the context of CSF for hexavalent chromium, EPA is in a position to conduct a more thorough and comprehensive assessment by including multiple endpoints, different model forms that allow for nonlinear dose-response, various BMR levels. The outcome will then demonstrate a range for POD and CSF to permit a better quantification and better understanding of uncertainties and variations.

Appendix B: List of Reviewers

United States
Environmental Protection Agency
National Center for Environmental Assessment

Peer Review Workshop for EPA's Draft Toxicological Review of Hexavalent Chromium

Hilton Crystal City Hotel
Arlington, VA
May 12, 2011

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Appendix C: List of Observers

United States
Environmental Protection Agency
National Center for Environmental Assessment

Peer Review Workshop for EPA's Draft Toxicological Review of Hexavalent Chromium

Hilton Crystal City Hotel
Arlington, VA
May 12, 2011

Final List of Observers

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Appendix D: Agenda

United States
Environmental Protection Agency
National Center for Environmental Assessment

Peer Review Workshop for EPA's Draft Toxicological Review of Hexavalent Chromium

Hilton Crystal City Hotel
Arlington, VA
May 12, 2011

Agenda

8:00 a.m. Registration/check in

8:30 a.m. **Welcome, Introductions, Meeting Purpose & Agenda**.....*Jan Connery, ERG (contractor)*

8:40 a.m. **EPA Welcome Remarks** .....*Vincent Cogliano, IRIS Program Director, EPA NCEA*

8:45 a.m. **Public Comment** ..... *Jan Connery*

9:30 a.m. **General Questions**..... *Anatoly Zhitkovich (Chair) & Panel*

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazard?

G2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

10:30 a.m. .....BREAK -

Chemical-Specific Charge Questions

10:45 a.m. **(A) Oral Reference Dose (RfD) for Hexavalent Chromium**..... *Anatoly Zhitkovich & Panel*

A1. A two-year drinking water study of sodium dichromate dihydrate in rats and mice (NTP, 2008) was selected as the basis for the derivation of the RfD. Please comment on whether the selection of this study as the principal study is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

A2. Diffuse epithelial hyperplasia in the duodenum of female mice was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

A3. Benchmark dose (BMD) modeling was applied to the incidence of diffuse epithelial hyperplasia in the duodenum of female mice to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for

use in deriving the POD (i.e., a 10% increase in the incidence of diffuse epithelial hyperplasia) scientifically supported and clearly described?

A4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically supported and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

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1:00 p.m. **(B) Carcinogenicity of Hexavalent Chromium**..... *Anatoly Zhitkovich & Panel*

B1. Under EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgrd.html), hexavalent chromium is *likely to be carcinogenic to humans* by the oral route of exposure. Is the cancer weight of evidence characterization scientifically supported and clearly described?

B2. A mutagenic mode of carcinogenic action by all routes of exposure is proposed as the primary mode of action for hexavalent chromium. Please comment on whether this determination is scientifically supported and clearly described. Please comment on data available for hexavalent chromium that may support an alternative primary mode of action.

B3. A two-year drinking water study in rats and mice (NTP, 2008) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantification is scientifically supported and clearly described. Please identify and provide the rationale for any other studies that should be considered.

3:00 p.m. BREAK

3:15 p.m. **(B) Carcinogenicity of Hexavalent Chromium** *(continued)*..... *Anatoly Zhitkovich & Panel*

B4. The incidence of adenomas and carcinomas combined in the small intestine of male mice from the NTP (2008) two-year drinking water study were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically supported and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

B5. The oral slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk of tumors of the small intestine in male mice). Has the modeling been appropriately conducted and clearly described?

4:30 p.m. **Reviewer Final Comments**..... *Anatoly Zhitkovich & Panel*

4:55 p.m. **Closing Remarks**..... *Jan Connery*

5:00 p.m. ADJOURN