SCIENTIFIC REVIEW OF TOXICOLOGICAL AND HUMAN HEALTH ISSUES RELATED TO THE DEVELOPMENT OF A PUBLIC HEALTH GOAL FOR CHROMIUM(VI)

Report Prepared by the Chromate Toxicity Review Committee

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EXECUTIVE SUMMARY

The committee considered all of the questions in our charge, reviewed the pertinent scientific literature, and was able to draw several conclusions with respect to the proposed PHG for Cr(VI) that has been recommended by OEHHA. OEHHA based their recommendation upon a specific animal study performed by Borneff et al. in 1968. The study used by OEHHA to develop this risk assessment, Borneff et al. (1968), is not suitable for use as the basis for a quantitative risk assessment for several reasons, as detailed in the report. We found no basis in either the epidemiological or animal data published in the literature for concluding that orally ingested Cr(VI) is a carcinogen, and a relatively large number of negative studies by the oral route of exposure, even at concentrations in excess of current MCLs. Definitive data on the potential carcinogenicity of orally ingested Cr(VI) should be provided by a planned NTP study, but these results will not be available for several years. While the regulatory agencies wait for these results to perform a definitive risk assessment, we would suggest that the current California MCL for total chromium of 50 ppb should be deemed protective of human health. Additional studies of the relative abundance of Cr(VI) in California drinking water supplies should be performed, with special emphasis on testing and validation of currently approved EPA standard methods, which may not be reliable when sampling procedures are not scrupulously controlled.

CHAPTER 1: THE OEHHA RISK ASSESSMENT

Quality and Result

The risk assessment performed for chromium(VI) by the Office of Environmental Health Hazard Assessment (OEHHA) of the State of California's Environmental Protection Agency was based upon the results of a study performed in mice, which was published more than 30 years ago (Borneff et al, 1968). The Borneff et al. (1968) study found excess tumors, predominantly papillomas of the forestomach, in female mice that drank water containing 500 mg/liter of potassium chromate (K_2CrO_4). Based upon this finding, OEHHA calculated a cancer slope value for chromium(VI) in mice, extrapolated this number to humans using various assumptions and established risk assessment methods, and used the resulting number to calculate a Public Health Goal (PHG) for chromium(VI) in drinking water. The result of these calculations was a proposed PHG of 0.2 ug/liter (0.2 parts-per-billion [ppb]). This may be contrasted with the current California State Maximum Contaminant Level (MCL) for total chromium in drinking water of 50 ug/liter (50 ppb) and the corresponding Federal standard of 100 ppb, based on a no observable adverse effect level (NOAEL) endpoint (MacKenzie et al., 1958). The MacKenzie et al. (1958) paper reported on a chronic drinking water study in rats where animals were given chromate in drinking water at six different concentrations between 0.45 and 25 parts-per-million (ppm) for 1 year. No toxic symptoms were observed at any concentration tested as based upon microscopic analysis of selected tissues, analysis of blood, or of animal body weight. OEHHA further assumed that 7% of total chromium in drinking water is in the valence state of Cr(VI) (based upon a single study of chromium ratios in water from two lakes in North Carolina). If this assumption is correct, the difference between the current MCL and the proposed new PHG would be 50 X 0.07 = 3.5 ppb Cr-6 versus 0.2 ppb, or about 17.5 times lower. If we note current reports of groundwater in certain California cities containing more than 50% Cr(VI), then the difference between the current MCL and the proposed PHG would be 50 X 0.5 = 25 ppb (or more) versus 0.2 ppb, or more than 125 times lower. The difference between the proposed new PHG and the current MCL hinges on whether Cr(VI) should be regulated as a carcinogen or a non-carcinogen in drinking water, and the quantitative basis for regulation as a carcinogen is derived from the Borneff et al. study. Thus, it is critically important to examine the quality of the data in the Borneff et al. (1968) study to ascertain whether it is an appropriate data set for performing the risk assessment for determination of a PHG for Cr(VI). It is also important to examine the actual risk assessment performed by OEHHA to ensure that their conclusions based upon the Borneff et al. study are consistent with proper handling of the data from this study.

Important Assumptions

There are, of necessity, several assumptions that must be made to perform a risk assessment for a suspected chemical carcinogen. Some of these assumptions are common to all risk assessments, while some relate to data gaps or other considerations peculiar to the specific studies used for establishing PHGs for specific compounds. In the risk assessment for Cr(VI), there are numerous issues that arise with respect to the Borneff et al. (1968) study that will be discussed in detail in the next chapter of this report. In addition, we will also examine the assumptions made by OEHHA to calculate a PHG from this data set. One major assumption is that the limitations of the cited study do not preclude these data from being used for a risk assessment. Additional major assumptions include that papillomas of the forestomach in mice are appropriately considered as tumors for the purpose of an animal bioassay for carcinogenesis and that female mice constitute a susceptible population. In addition, no consideration was given in this risk assessment to the issue of whether the toxicokinetics of Cr(VI) ingested in water by

mice, which have an anatomical entity with no human analog, the forestomach, allows for simple extrapolation to human dose.

Problems Identified

There are some specific problems with this risk assessment as it was performed. The major problem with the Borneff et al. study was a serious outbreak of disease (ectromelia, or mouse pox) in the mice being tested, which killed a high percentage of the animals during the first year of this study. Only a fraction of the original mice survived as the test animals. There were three different generations of mice studied, and data from all three generations were inappropriately combined for the OEHHA analysis. There was only one dose used in the study. The mathematical model used for conversion of the dose-response data to a cancer slope factor assumes response to a range of concentrations below the maximum tolerated dose (MTD), which was not the case for this study, which was performed at the MTD (Borneff et al., 1968). This would have an impact on the precision of determining the correct concentration to identify as causing a risk of 1 in a million, and on the uncertainty of this estimate. According to most guidelines, if a study must rely only on data obtained at the MTD, it is not appropriate for use in quantitative risk assessment (e.g., dose-response modeling). The proposed PHG fails to indicate any level of uncertainty, and obscures details of the assumptions and calculations made by citation of a proprietary computer model used. This lack of explicit definition of uncertainty seriously compromises the process of risk management, and can also unnecessarily alarm the public when discordant values are identified between the PHG and MCL. As a minimum, it would be useful for the State to present all of the modeling results (lower and upper confidence limits, and maximum likelihood estimate (MLE). In addition, the water given to the mice in the Borneff et al. (1968) study contained a detergent to help solubilize the added compounds; this introduced an “unnatural” compound into the mice which could confound the interpretation of these studies.

OEHHA chose to examine only data from the female mice in this study, defining them as a "susceptible population" because 11 of the total of 12 tumors found in the chromate exposed mice were in the females. However, this is not a statistically or scientifically sound definition. Nine of these tumors were found in the F0 generation mice. The exact gender distribution of these tumors in the 41 surviving F0 mice is unknown, but either 9 females or 8 females and 1 male had tumors. At the start of the experiment there were 120 female and 10 male mice in the F0 generation exposed to chromate. If female and male mice were equally susceptible to any putative carcinogenic property of chromate, then we would expect to find tumors in a ratio of 12:1 favoring the F0 females by chance alone. Therefore, the apparent higher incidence of tumors in surviving females in this experiment merely reflects the size of the initial population at risk, and does not define females as a "susceptible population" to a carcinogen.

Significance of Papillomas in the Mouse Forestomach

Several additional experts in laboratory animal medicine and veterinary pathology were consulted about mouse pox by the panel. We inquired specifically about the lesions that are found in affected animals. There are several aspects that are noteworthy. The progression of pox lesions in the skin follow a classic “pox virus” progression from vesicle to necrosis and ulceration and regenerative healing (if the mouse survives). In the healing phase there can be exuberant proliferation of squamous epithelium, which may mimic that of a squamous papilloma, not dissimilar to what was described in the forestomach in the Borneff study. Also, importantly, mouse pox is caused by a polytropic virus; thus, there are lesions in multiple tissues/organs including the oral cavity and intestinal tract in addition to the cutaneous ones.

The description of "sarcomatous" change is highly unusual in forestomach tumors. This component of the lesion could be a highly proliferative inflammatory lesion; aggressive ulcerative reactions in the skin often resemble fibrosarcomas, particularly during the healing/regenerative phase. Whatever the correct interpretation, the ambiguity introduced by the mouse pox epidemic in the Borneff et al. study is adequate and sufficient to render these data unsuitable for use in a carcinogenesis risk assessment. These concerns underlie the rationale for the National Toxicology Program (NTP) and Environmental Protection Agency (EPA) guidelines that prohibit use of data from infected animals for such a risk assessment.

We have looked at numerous studies where forestomach tumors were part of the response to the xenobiotic. In those cases, most, if not all, of the animals in the affected exposure group also showed squamous hyperplasia/hyperkeratosis. This was not found in the F1 and F2 animals in the Borneff et al. study, and "it was looked for" - see Table 3. If Cr(VI) is a forestomach carcinogen, it should have caused such lesions in a majority of the animals not just in the F0, but also in the F1 and F2 generations**,** particularly in light of the fact that the F1 and F2 mice were exposed to the same concentration of Cr(VI) as the F0 animals.

Toxicokinetics of Cr(VI) in the Stomach

The OEHHA Draft PHG for chromium in drinking water includes an implicit uncertainty factor for interspecies extrapolation from mice to humans. Usually a default factor of 10 is used to "correct for" the hypothetical potential for humans to be more sensitive to a putative carcinogen than mice. However, special considerations pertain in this specific risk assessment that make such routine use of an uncertainty factor of 10 for mouse to human extrapolation questionable. The pH of the human stomach is strongly acidic, of the order of pH 1-1.5 in normal individuals. The potential issue of "sensitive populations" will be discussed separately below. Mice have a special organ, the forestomach, for which there is no human analogue (the forestomach perhaps is best compared to a pouch in the esophagus, as opposed to a true stomach). The pH of the forestomach is neutral, approximately 7. Thus, chromate ingested by the mice in the Borneff et al. study had the potential to remain at a neutral pH, and therefore at a valence state of Cr(VI), while in the forestomach, which was the site at which tumors were observed in this study. Humans ingesting Cr(VI) in drinking water, on the other hand, would immediately subject the Cr(VI) to a strong reducing environment, the acid milieu of the stomach. Cr(VI) would be reduced to Cr(III) at a rapid rate, and the effective dose of Cr(VI) would be a lot lower than would be experienced by a mouse drinking the same water. It is difficult to calculate this effective dose precisely, as we know neither the exact rate of uptake of Cr(VI) into target cells in the (fore)stomach, nor the exact rate of reduction of Cr(VI) in the human stomach. The many in vitro experiments that have been published can, and do, demonstrate the principle. However, these in vitro experiments can not precisely define rates in vivo, which would depend on additional factors than the pH per se [for example, the availability of chemicals that can serve as electron donors to allow the reduction of Cr(VI) to Cr(III)]. However, it is clear that based upon current knowledge, a physiologically based pharmacokinetic approach to comparison of the mouse forestomach to the human stomach should demonstrate that the mouse is the more susceptible species to Cr(VI)-induced tumors. Therefore, the uncritical application of an uncertainty factor of 10 to correct for comparative sensitivity that assumes humans are more sensitive than mice is not sound science. Given the present non-definitive state of quantitative knowledge of rates of reduction of Cr(VI) in the stomach versus rates of intracellular uptake and metabolism to toxic species, the best approach is probably to eliminate the use of any uncertainty factor for interspecies extrapolation in this calculation rather than to attempt a quantitative estimate of relative sensitivity. Thus, were one to use the data from the female mice in the Borneff et al. study, the estimate of cancer potency (or cancer slope factor) used by OEHHA

should probably be adjusted downward by a factor of 10 for calculation of the PHG to conservatively account for interspecies extrapolation.

The Panel's Views of the Conclusions

We conclude that there is a high probability that the forestomach lesions in the F0 mice in the Borneff et al. study were related to the mouse pox pathological syndrome. No excess tumors were found in the F1 and F2 generations exposed to the same concentrations of Cr(VI); the infection was completely controlled in the F2 mice by vaccination of the F0, F1 and F2 animals.

We also conclude that the OEHHA risk assessment was not in concert with the statutory language in SB 635. SB 635 requires that "The risk assessment shall be prepared using the most current principles, practices, and methods used by public health professionals who are experienced practitioners in the fields of epidemiology, risk assessment, and toxicology." Contemporary practice in risk assessment would immediately discard data from any study where the animal health status was as compromised as in the Borneff study by an intercurrent outbreak of a highly lethal systemic disease such as mouse pox.

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CHAPTER 2: THE BORNEFF et al. STUDY

Description of the Study

Swiss-Webster mice were given drinking water containing either Prell shampoo (controls) or Prell shampoo + 550 mg/L Cr(VI) (experimental animals) for a total of 880 days. This was part of a larger experiment that also looked at co-carcinogenesis of Cr(VI) in mice administered 3,4-benzpyrene in their drinking water. Initially, there were 480 female and 40 male mice in the study; each experimental group contained 120 females and 10 males. These animals were bred to give an F1 generation of mice, which was added to the experiment. Eight months into the study, an outbreak of ectromelia (mouse pox) killed 512 of the mice in the study; the remaining mice were administered a vaccine against mouse pox. An additional 69 mice died, but the epidemic of mouse pox in this colony was halted. Additional vaccinations were given to the surviving animals and their subsequent offspring, the F2 generation. A total of 126 mice (79 female, 47 male) survived in the control group (pooled F0, F1, and F2), and 101 mice survived in the group drinking water containing Cr(VI) (66 female, 35 male). For the control group 54 of the surviving mice were F0 generation, 24 were F1, and 43 were F2, while for the group exposed to Cr(VI), 32 of the surviving mice were F0 generation, 21 were F1, and 36 were F2. The excess stomach tumors reported (papillomas + carcinomas + hyperkeratomas) were all found in female mice in the F0 generation (9/41 versus 2/56 in the controls; see Table 3). There was no increase in stomach tumors in either sex in the F1 or F2 generations.

Forestomach Tumors in the Cr(VI) Treated and Control Groups: [The following table is adapted from Table 3 of the Borneff et al, 1968 report.]

Group	(n)	Forestomach tumors
FO Controls	56	2 (3.6%)
FO K2CrO4	41	9 (22%)
F1 Controls	25	1 (4.0%)
F1 K2CrO4	22	1 (4.5%)
F2 Controls	45	2 (4.4%)
F2 K2CrO4	38	2 (5.3%)

Conclusions of the Authors

The authors conclude, and we quote (their translation): “The evaluation of the weight curves, the survival times, the tumour types and rates and the concentrations of cancerogenics did not permit to draw the binding conclusion that chromate has cancerogenic effects in the mouse stomach. 2 carcinomata in 101 animals (2 years daily feeding with approx. 1 mg K_2CrO_4) gave rise to further tests. An increase of the rate of benzopyrene-induced gastric tumours by chromate could not be achieved in the meaning of the syncarcinogenesis. An ectromelia epidemic is considered a possible cause of the delayed tumour formation in one of the test groups.”

Problems Identified by the Panel

1. The most important problem in the study is the ectromelia (mouse pox) outbreak. Most of the F0 and F1 mice in this study died from this infection. From Figure 2 it can be concluded that the mortality rates in the control group and in the group given chromate in the drinking water were approximately 60% to 70% in both the F0 and F1 generations. This, in itself, makes these two parts of the study of no value for identifying carcinogenic activity of this or any other xenobiotic. In contemporary studies, one would discard all of the mice and start the study over with "clean" animals. Vaccination is not an acceptable alternative.
2. Housing 10-12 mice/cage invites many problems, e.g. fighting, competition for food, etc. This is another source of stress for these animals, which may be reflected in the low fecundity (see below), as well as the outbreak of ectromelia.
3. Borneff et al. report 1105 offspring from the 480 F0 females, which means 2.3 pups/litter. This is unacceptably low for most strains of mice, including the Swiss-Webster mice used in this study. It is probable that many of the offspring were cannibalized due to the multiple housing used in the study, although this can not be ascertained with certainty. However, it does explain why only 2 pups/litter were used for the F1 generation. Note that there is no mention of how the animals were chosen for placement into exposure groups, so randomization may have been an issue. The number of pups surviving from the 1st generation dams (F2 pups) was even worse, i.e. 364 pups from 220 females (1.7 pups/litter). Again, we feel the most reasonable explanation for the low fecundity is cannibalism after birth.
4. The high degree of cannibalism suggests that the animals were not adequately monitored during the course of the study. However, the multiple housing certainly exacerbated this situation. The authors' argument that cannibalism was the result of the mice preferring moisture from consuming their cage mates to the Cr(VI) water is probably true. The authors also note "We chose 550 ppm for our potassium-chromate concentration, a quantity that can barely be tolerated without damage by mature white mice." Mice (especially males) are typically highly aggressive in multiple housed caging. This is exacerbated when they have cutaneous lesions, which invite biting, as would be the case with mouse pox.
5. The body weight curves (available in the original paper in German) allow us to calculate the amount of Cr(VI) ingested on a body weight basis. If we assume a body weight of 30 grams on average throughout the study, this would work out to about 33 mg/kg/day. This information is important in the context of the doses used in this study as they relate to the estimated human exposure, which is 0.0048 mg/Kg/day at 67 ppb, which is slightly above the California MCL of 50 ppb [2.4 mg/Kg/day NOAEL / 500 (total uncertainty factor)].
6. Chromium levels measured in mouse tissues were inconsistent in the Borneff et al. study (their Table 5; note for example the apparent factor of 5 difference in chromium content of lungs in group 2 and group 4 mice receiving the same total dose of chromium in the study).

Comments on the Authors' Interpretation of their Study

1. The authors' interpretation was appropriately conservative, except for the fact that they should have totally discounted the F0 and F1 generation results. If this is done, there is no evidence of carcinogenic activity in this study. However, the F2 generation study is also confounded because it may not have been of long enough duration (17 months) to find a carcinogenic effect.

There are other problems in the Borneff et al. report that make interpretation difficult:

1. It appears that the authors combined hyperkeratoma, papilloma, and carcinoma for determining the carcinogenic potential. This is appropriate for papilloma and carcinoma, but not for hyperkeratoma unless it is truly a neoplasm and not merely hyperkeratosis. This problem is further compounded by the fact that, in the original paper, the text refers to hyperkeratosis (pg. 49, line 7), a proliferative non-neoplastic lesion, whereas the title of Table 3 lists "Hyperkeratome", which denotes a neoplasm. If hyperkeratoma equals hyperkeratosis, then it should be deleted from a tumor effect. It is impossible to determine if this is the case. However, one can assume that the authors felt that these lesions were "different" or they would not have used both terms. The other side of the argument is that they felt that while they were morphologically different, they were both neoplasms. The importance of this finding is that if this is indeed hyperkeratosis rather than a neoplasm, then the number of tumors in the Cr(VI) females in the F0 generation may not be different from the controls. It is impossible to determine the mix of hyperkeratomas, papillomas, and carcinomas in the F0 generation; the only generation showing a possible carcinogenic effect.

   Another interesting observation is that epithelial hyperkeratosis or hyperplasia was not increased if hyperkeratoma is indeed a tumor. The importance of this observation is that forestomach carcinogens are invariably associated with a concomitant increase in the incidence of hyperkeratosis in that exposure group. Forestomach carcinogens are direct acting and hyperkeratosis/hyperplasia always precedes, and is always found in the presence of, forestomach neoplasms. Since forestomach hyperkeratosis/hyperplasia were not found, one has to question the relevance of the reported increase in forestomach tumors, at least in regard to causation by Cr(VI) or any other xenobiotic.

2. The authors differentiated total number of tumors in each generation in Table 3, but they did not do this in Table 2. As a result, we do not know if the two carcinomas (Table 2) occurred in the F0, F1, or F2 generation, or if one was found in each of two generations.

3. The authors combined the results of all three generations to determine a carcinogenic effect. This is not appropriate. Each generation should be considered a separate study. The primary reason for this is that although the concentration of chromate given to all three generations is the same, the exposure each received is actually quite different. The exposure in the F0 generation was only during "adulthood", while that of the F1 and F2 included in utero, lactation, infant, and adult exposures. This is one of the more perplexing aspects of this study. Intuitively, one would expect that the F1 and F2 generations would have a greater chance of showing a carcinogenic response than the F0 generation because of the greater total exposure and, most importantly, during critical times of organogenesis. The authors also combined males and females, which should not be done unless there are defined reasons for doing so, i.e. that males and females are comparable in their response, which may not have been the case in this study. However, this is not a major confounder because so few tumors were found in the males.

How the Borneff et al. Study has been used by Agencies

Except for OEHHA, other regulatory agencies attempting to analyze the risk of ingested Cr(VI) have deemed this study not suitable for use as the basis for a risk assessment and have not used these data to estimate a cancer risk. OEHHA has selectively taken the data for the female mice in this study, in essence choosing the data from the F0 generation only, and used these results to perform a risk assessment. They fail to mention in their risk assessment the negative results in this study with the F1 and F2 generations, or the potential confounding effects of

mouse pox on these data. They also fail to mention any of the other assumptions that we have discussed in the Borneff et al. study.

Suitability of this Data Set for Risk Assessment

There are in reality three studies reported by Borneff et al. The F0 generation shows a definite carcinogenic response, if the reported tumors are in fact neoplasms and not examples of hyperkeratosis. However, the other two studies (F1 and F2) show no carcinogenic effect, even though these mice had a greater exposure, i.e. for their entire life compared to adult only in the F0 generation.

We are also left with the confounding effect of the pox virus infection, the impact of which on the forestomach is unknown. We do not know if poxvirus, in and of itself, could cause forestomach lesions. The reason for this is that when a mouse pox infection occurs, the colony is typically destroyed and the long-term effects of the virus are not investigated. However, this study does shed some light on this issue. The F1 generation also had the infection but did not show a tumor response. Therefore, we can assume that the poxvirus was not the sole cause of the observed forestomach tumors unless one assumes that in utero and perinatal exposure to Cr(VI) was somehow protective of the forestomach response. We find no scientific justification for this assumption.

Finally, the 3,4-benzpyrene portion of the study does not help us much either. The primary reason is that the tumorigenic response in the F0 and F2 generations was so high that it precluded finding any additive or synergistic effect. However, the F1 generation results may aid in this matter. The incidence of forestomach tumors in the 3,4-benzpyrene alone group was 38%, compared to 30% for 3,4-benzpyrene plus Cr(VI). This incidence is certainly low enough to determine an additive or synergistic effect if one existed, which was not observed. However, this conclusion is clouded by the marked generational difference, i.e. the incidences in the F0 and F2 generations were markedly higher than the F1.

In summary, we feel that the confounding nature of the reporting, the presence of the pox virus infection resulting in marked mortality, and conflicting results of these three studies of Cr(VI) preclude us from using the findings of this study for determining the carcinogenic effects of Cr(VI) from oral exposure. This said, we feel just as certain (as did the authors of the study), that the results of this study do not indicate that Cr(VI) administered in drinking water has carcinogenic activity in this strain of mouse.

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CHAPTER 3. OTHER ANIMAL STUDIES AND THEIR SUITABILITY FOR RISK ASSESSMENT

Description of Key Studies

A few shorter-term studies of ingested Cr(VI) and Cr(III) have been conducted that might help in the interpretation of whether Cr(VI) is a carcinogen when ingested orally. Meenakshi et al. (1989) exposed male rats for 60 days via gavage to 10 mg Cr/Kg/day as solutions of Cr chloride [Cr(III)] or potassium dichromate [Cr(VI)]. Hepatic and renal effects were reported. MacKenzie et al. (1958) exposed rats to 0.45, 2.2, 4.3, 7.7, 11, or 25 ppm of Cr(VI) in drinking water for up to 12 months. After one year, rats in the three lowest dose groups did not show appreciable accumulation of Cr in the liver. An increased concentration of Cr was found in all tissues examined in animals receiving 7.7, 11, or 25 ppm of Cr(VI). Limited histopathology was performed; all measures of putative liver and kidney damage were consistent with controls. Stomach tumors or papillomas were not reported.

The National Toxicology Program (NTP, 1996a,b, 1997) conducted a series of studies in which Cr(VI) was administered orally to Sprague-Dawley rats and BALB/c mice for up to nine weeks. The studies were designed to evaluate the potential for reproductive and developmental toxicity; however, histopathology and blood chemistry were also examined to observe effects outside of the reproductive system. In the initial study (NTP, 1996a), groups of male and female rats were administered potassium dichromate in their diet at concentrations of 15, 50, 100, and 400 ppm for nine weeks, and were followed for an eight-week recovery period. The equivalent dose levels are 1, 3, 6.5, and 26 mg/Kg-body weight per day (mg/Kg-day), respectively. Necropsies were taken after 3, 6, and 9 weeks of exposure, and a final necropsy was taken at week 17. No significant exposure-related effects were observed in the rats. At the highest dose, significant decreases in the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were observed in both males and females, but the MCV and MCH values returned to normal during the recovery period. In the second study (NTP 1996b), male and female mice were administered potassium dichromate in the diet at the same concentrations as in the first study. The resulting doses were 4, 13, 28, and 115 mg/kg-day, respectively. Necropsies were taken at the same time intervals as in the first study. Again, no significant treatment-related effects were observed; however, at the highest exposure level, both MCV and MCH were depressed. A third multigenerational study was also performed, but treatment for this study lasted for only two weeks (NTP 1997), and no treatment-related effects, other than those previously noted, were observed.

Grosse and Heller (1946) exposed white mice, albino rats, and New Zealand rabbits to high levels of Cr(VI) in drinking water (up to 500 ppm) and feed (up to 10,000 ppm) for 10 months, and the general health of the animals was recorded. It was reported that up to 500 ppm and 300 ppm Cr(VI) in drinking water generated no abnormal characteristics in the mice and rats, respectively. At 500 ppm in the rats, “slight roughness of the coat” was reported. More severe effects, including sterility, were observed in rats administered Cr(VI) in their diet at exposures starting at 1,250 ppm as zinc chromate. Anwar et al. (1961) exposed two dogs to Cr(VI) at 11.2 ppm in water for 4 years. Based on urine analysis and gross and microscopic examination of the major organs, no adverse effects were noted (Anwar et al., 1961). Finally, Maruyama (1982) exposed mice to water containing 25, 50, and 100 ppm of Cr(VI) for one year. No adverse effects were reported based on the results of hematological and biochemical analyses. The 25 and 50 ppm dose groups gained more weight than the controls, but weight gain

among the 100 ppm exposure group was consistent with the controls. The authors reported Cr accumulation in all organs following administration of Cr(VI); however, after exposure to Cr(III) under the same conditions, total Cr concentrations were elevated only in the liver.

Genotoxicity of Chromium(VI) in vivo

Cr(VI) is clearly genotoxic to cells in culture, as is supported by a large literature (DeFlora, 2000). Inhaled Cr(VI), at least in certain chemical forms, is clearly carcinogenic and genotoxic. However, there is controversy as to whether ingested Cr(VI) is genotoxic to humans and animals. It should be noted that most of the reports of positive genotoxicity by the oral route involve administration of large bolus doses of Cr(VI) by gavage, an unphysiological route of administration that could have transiently overwhelmed the reductive capacity of the stomach and would not be directly relevant to ingestion of low levels of Cr(VI) in drinking water. A review by Cohen et al. (1993) and a more recent review by Costa (1997) are frequently cited as references for the genotoxicity of Cr(VI) in vivo. The former paper, however, carefully distinguishes between genotoxicity of Cr(VI) that has been demonstrated in cultured cells, genotoxicity after inhalation and instillation into trachea, pleura, and bronchus, and genotoxicity after systemic injection. Issues of putative effects after oral ingestion are discussed mainly in the context of reduction of Cr(VI) to Cr(III) in the stomach resulting in a low systemic uptake of Cr by absorption (typically 1-3% of the total dose). The latter paper attempts to demonstrate from epidemiological studies that Cr(VI) can cause cancer at various sites throughout the body, regardless of its route of entry. This review and its conclusions are criticized strongly by DeFlora (2000), and the correct interpretation of these studies remains a subject of controversy. DeFlora (2000) specifically suggests that "oral chromium is not genotoxic at doses which greatly exceed the drinking water standards", and cites studies where a bolus dose of 5 mg of Cr(VI) in drinking water did not increase DNA-protein crosslinks in white blood cells in humans, nor did a dose of 20 mg Cr(VI) in drinking water elicit genotoxic effects in mice (both male and female mice were tested) or rats. He argues for there being a threshold mechanism in Cr(VI) carcinogenesis (and genotoxicity), based upon the capacity of mammals to detoxify Cr(VI) by reduction to Cr(III). These arguments have been accepted by International Agency for Research on Cancer (IARC), Agency for Toxic Substances and Disease Registry (ATSDR), and the U.S. EPA as the basis for their choice not to regulate Cr(VI) in drinking water as a carcinogen.

Conclusions

The animal data that are available to evaluate the carcinogenicity of Cr(VI) are not definitive, because most studies looked at effects after less than lifetime exposures and evaluated an incomplete set of possible endpoints. The existing data from the chronic bioassays (up to 1 year of exposure) do not indicate that Cr(VI) poses a cancer hazard via ingestion. Nor can they prove conclusively that it does not. Data as to whether ingested Cr(VI) is genotoxic in vivo are equivocal, and have been interpreted to suggest that there may be a threshold for such effects (DeFlora, 2000). Four chronic drinking water studies (Anwar et al., 1961; Grosse and Heller, 1946; MacKenzie et al., 1958; Maruyama, 1982) and four subchronic oral studies (Meenakshi et al., 1989; NTP, 1996a,b; 1997) reported no evidence of carcinogenicity. However, these studies were not designed to observe a carcinogenic endpoint in the digestive system. The only study that did look at lifetime exposure, Borneff et al. (1968), is confounded by an infection of mouse pox virus, and the conflicting nature of its results. It should be noted that all of the studies cited administered Cr(VI) at levels that far exceed (more than 100-fold) the current Federal and California drinking water standards.

Relevance of other Animal Studies to the Borneff et al. Study

One of the basic tenants of toxicology is "reproducibility", i.e. consistency of results. In that context the Borneff study is clearly an "outlier", notwithstanding the fact that no other long-term carcinogenicity studies have been conducted with orally ingested Cr(VI). First, as noted above, hyperplasia and hyperkeratosis invariably are associated with, and precede, the development of forestomach papillomas. Hyperplasia and hyperkeratosis are generally found within 90 days, and often within 30 days, of treatment with a forestomach carcinogen. However, no such lesions were found in mice or rats at Cr(VI) doses similar to, or higher than, those used by Borneff et al. In the study by the NTP, concentrations in the feed were 400 ppm in rats (26 mg/kg) or in mice (115 mg/kg) for 9 weeks. Grosse and Heller (1946) examined water at 500 ppm chromate for 10 months in rats or mice, and Maruyama (1982) water at 100 ppm of chromate for one year in mice. If Cr(VI) is capable, in and of itself, of causing forestomach lesions in mice, one or more of these other studies probably should have shown squamous cell hyperplasia/ hyperkeratosis. Therefore, the forestomach tumors reported by Borneff, if they are truly neoplasms, should be not be viewed as a response to Cr(VI).

How the Animal Studies Cited have been used in Risk Assessments

Except for the proposed use of the Borneff et al. study as the basis for a PHG by OEHHA, none of these studies have been used in carcinogen risk assessments for the reasons discussed above. MacKenzie et al. (1958) was the source of the data for a non-carcinogen risk assessment to set the current MCL both by California and the U.S. EPA.

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CHAPTER 4: HUMAN STUDIES OF THE CARCINOGENICITY OF CHROMIUM(VI)

Description of Key Studies of Chromium(VI) in Drinking Water

Two types of epidemiologic studies provide data on the possible carcinogenicity of ingested chromium—studies of chromium in drinking water and occupational cohort studies of inhaled chromium. Studies of human populations who have directly ingested chromium in drinking water provide direct data on potential health risks, and will be reviewed in some detail. There have been many occupational cohort studies of inhaled chromium, but they can provide only indirect evidence of health risks from ingested chromium, and will only be summarized here.

Zhang and Li reported on the effects of drinking water containing chromium from wells contaminated by effluent from a chromium alloy plant (Zhang and Li, 1987). Chromium smelting began in 1961, at which time contamination of underground water began. The plant began regular production by 1965, by which time 28% of the wells were severely polluted, with 55% of the contaminated wells having Cr(VI) concentrations of greater than 20 mg/L. A contamination zone of 22.5 km^2 spread from the factories. Concentration of Cr(VI) in a major reservoir was 0.004 mg/L (4 ppb). A dumpsite of 300,000 tons of dregs from the plant may have been a source of continuing release of chromium. It was estimated that as much as 0.3 ton of hexavalent chromium was released into underground water from the dumpsite. Hexavalent chromium was detected in the dump site soil at concentrations as high as 4700 mg/kg. In 1980-82, some 20 years later, an underground prevention system was applied with gradual reduction in underground water pollution. A cross-sectional survey was completed in 1965 on 155 villagers who had drunk water from wells with chromium levels of $\geq$20mg/L. An association of several symptoms (oral ulcer, diarrhea, abdominal pain, dyspepsia, and vomiting) and laboratory results (leukocytosis and immature neutrophils) with chromium is reported, but the data for a reference population for this conclusion is not indicated.

A retrospective mortality study was conducted on the population who lived in the polluted zone. Data from 1970–1978 revealed excess malignant neoplasm rates (71.9–92.7/10^5) compared to the general surrounding district (65.4/10^5). Adjusted mortality rates for stomach cancer were above the average level for the whole district. The paper provides no indication of what, if any, adjustments were made to the rates for age or socio-economic status (SES), factors that have a strong effect on cancer rates. A reported difference in lung cancer mortality rates suggests that there were clinically significant differences in the populations compared. It is also not indicated if the comparison was of two indirectly standardized rates (presumably done because of small numbers of cases), or adjustment to a common reference population. No statistical tests of the comparison are reported. The reported "association" of chromium in water and various symptoms and pathologic findings is difficult to interpret in the absence of more information on how the data were collected, what comparison rates were used, and how various biases were controlled.

Ten years later these authors reported a follow-up to this study (Zhang and Li, 1997). This follow-up suggested that the difference in overall cancer mortality in the earlier report was significant ($p=0.04$) in the five Cr(VI) contaminated villages combined compared to the surrounding district. In this later report the authors analyzed overall, lung and stomach cancer mortality rates for the same years (1970–1978). Rates were calculated for the six villages most

highly contaminated with Cr(VI), and for the six suburb regions in the district. None of the individual villages had a statistically significant difference in cancer rate (overall, lung, or stomach) from the surrounding province. Further, analysis of mortality rates for the same cancers in the individual villages found no suggestion of a dose-response, i.e. villages closest to the source of contamination did not have higher cancer mortality rates.

These papers thus provide no support for the hypothesis that high levels of Cr(VI) in drinking water are associated with an increase in stomach (or overall) cancer mortality. Some caution is necessary in this interpretation because negative results may have been due to the short latency period (1965–1978). The absence of additional information on the populations and what adjustments to data were done further limit interpretation of the results, although such information would have been more important if positive results had been reported. Data are not provided on other causes of mortality to help in the interpretation of these findings.

Epidemiologic studies conducted in the Leon valley of central Mexico (Armienta-Herrnandez and Rodriquez-Castillo, 1995) near the site of a chromium contamination are too limited in their analyses to determine whether there is an association of chromium ingestion and cancer. Concentrations of Cr(VI) in groundwater and in potable well water were as high as 60 ppm and 0.5 ppm, respectively. Ambient air concentrations were less than 25 $\mu g/m^3$ (the analytical limit of detection). Armienta-Hernandez and colleagues (1995) found increased concentrations of total Cr in the urine of residents living near the contaminated groundwater compared to a reference population (27.3 ±28 ng/mL exposed, 20 ±8.8 ng/mL reference group). The health component of the investigation consisted of a door-to-door survey of adverse health outcomes, including cancer. This ascertainment method provides no basis for estimating cancer rates and no useful epidemiologic data on the question of chromium ingestion and cancer.

In the town of Lecheria, in southern Mexico, approximately 3,000 residents were exposed to Cr(VI) in soil, drinking water, and air due to emissions from a chromite production plant (Rosas et al., 1989; Neri et al., 1982). The plant had been established in 1958 and used "old technology" until 1973. It was closed in 1978 because of public health concerns. Gross environmental contamination was suggested by the solid waste from the plant being used as street fill, and wet solid waste being left in open areas next to the plant. The concentrations of total chromium in groundwater used as drinking water was 0.9 ppm (900 ppb), and the concentrations in air were 0.25–0.39 $\mu g/m^3$ (Rosas et al., 1989). Concentrations of chromium in urine and hair in the exposed community were significantly elevated as compared to a control population (Rosas et al., 1989). There was no increase in the number of deaths due to cancer among the exposed population (18/947 deaths = 1.9%), as compared to the control population (39/1972 = 2.0%) over the 24 year period studied (Neri et al., 1982). This proportional mortality study with negative results did not give any information on age distribution between the two populations, potential chromium exposure of cases (e.g. closeness to the factory), or residence history. It is thus of minimal value in addressing the potential carcinogenic effect of environmental chromium exposure.

The Department of Health of the Greater Glasgow Health Board conducted a mortality assessment for residents of a part of Glasgow known to be contaminated with Cr(VI) from chromate slag in soil (GGHB, 1991). The source of the slag was a chromate production plant that operated from 1930 to 1963 in Glasgow. A cohort study of mortality among workers at the plant demonstrated excess lung cancer mortality (O/E = 75/26.3 = 2.85) (HSE, 1989). Concentrations of total chromium in the soil were reported "to be of the order of 10,000 ppm" (mg/kg). The concentrations of Cr(VI) were not specified in this report. There were six postal codes with known contamination, and several others with suspected contamination. Each postal

code had approximately 5,000 residents; thus, the size of the exposed population was potentially greater than 30,000. The areas with "known" contamination were evaluated separately from those with only "suspected" contamination, so the potentially exposed population was not biased with unexposed individuals. Using cancer registry data, which is kept by postal code, the researchers compared the observed all-cancer and site-specific cancer mortality rates in the affected areas with those expected on the basis of observed rates for the City of Glasgow. Deaths were analyzed for the period from 1975 to 1989. The investigators found no increase in the rate of total cancer mortality, lung cancer mortality, or cancer mortality of any other site among the potentially exposed population. Unlike the study by Zhang and Li (1997), in the Scottish cohort the latency is likely to have been at least 30 years—adequate to have observed an increased rate of cancer among the exposed population. However, there are no quantitative measures of exposure available for this population.

The Greater Tokyo Bureau of Hygiene (GTBH, 1989) studied a cohort of residents who lived in an area where the soil contained chromate production slag. The Cr(VI) content of the slag was not described, but the authors report that chromium dusts "accumulated at high concentrations," presumably in the homes. Multiple and highly detailed individual health studies of the residents in the affected neighborhoods were conducted at a local medical school, but mortality or cancer incidence was not investigated. This study thus provides no useful data on potential carcinogenesis of environmental chromium exposure.

A recent study reported on mortality of residents living near three Southern California gas compressor facilities (Fryzek et al., 2001). Soluble Cr(VI) had been used as cooling water tower additives from the 1950s to the 1980s at gas compressor plants in the three locations. Wastewater from the cooling towers was disposed of in ponds near the plants, with subsequent contamination of the water table. Deaths were abstracted from the California Death Statistical Master file, and population was based on 1990 US Census data. Comparisons were made to deaths occurring in the two counties in zip codes not in the vicinity of the three plants. The analysis was based on 107,227 deaths occurring between 1989 and 1998. The mortality rates of residents living near the three plants for all causes, lung cancer, and all cancers combined were not significantly different than rates for residents in the other areas of the counties. Similar results were observed when the results were analyzed by sex, except for an overall cancer mortality rate that was significantly lower among women living in the exposed areas. The findings of this study (see Table 1) are not surprising, since the authors had previously reported on a retrospective mortality study among PG&E workers at the same facilities and found no increase in all-cancer or lung cancer mortality (Blot et al., 2000). Occupational exposures are usually considered to result in higher exposures than environmental exposures.

The major limitation of this study is that it is an ecologic analysis. No individual data are available for specific individuals such as cigarette smoking or diet. Actual exposure to chromium is also unknown. Nevertheless, the study has many strengths including the large sample size, long latency period, and the systematic mortality ascertainment. This study thus provides the best epidemiologic data to date on potential carcinogenicity of environmental chromium exposure. It suggests, but does not prove, that environmental chromium exposure at levels that have occurred in California is not associated with a significant increase in overall cancer mortality. The study does not provide any data suitable for a quantitative risk assessment, although exposure data from the affected areas might be used to set a NOEL.

Conclusions

Taken together, the epidemiologic data on Cr(VI) exposure from environmental sources (as opposed to generally much higher occupational exposures) provide no support for a causal

association of exposure to Cr(VI) and overall or site-specific cancer mortality for the general public. In general, actual chromium exposure was unknown in these studies. Nevertheless, biologic markers in some of the studies demonstrated increased chromium exposure. It is not possible to compare exposures at the different sites, and no sites provided enough data to estimate a possible dose-response. Epidemiologic limitations in some of the earlier studies (short latency, lack of rate adjustments) were corrected in later investigations. The major limitation is that all of the investigations were ecologic in design. Thus, while a large increase in cancer risk upon exposure to Cr(VI) by ingestion at environmental concentrations can reasonably be excluded, this design does not permit the exclusion of a very small increase in site-specific cancer risk.

Table 1: Age-Adjusted Relative Mortality Rates for Residents in Three Chromium Contaminated Areas in California, 1989-1998

Outcome	Deaths in Exposed Areas	Relative Mortality Rate	95% CI
Lung Cancer			
Men	147	0.98	0.83-1.16
Women	97	1.06	0.87-1.30
Both	244	1.03	0.90-1.17
All Cancer			
Men	438	0.96	0.87-1.06
Women	327	0.87	0.78-0.97
Both	765	0.93	0.87-1.00
All Mortality			
Men	2045	0.97	0.93-1.01
Women	1604	0.98	0.93-1.03
Both	3649	0.98	0.95-1.02

Occupational Cohort Studies

Occupational cohort studies are the other epidemiologic approach that may provide information about cancer risk from chromium ingestion. Interpretation of these studies is complicated by lack of information on the actual amount of chromium ingested. The disposition of inhaled particles may be a function of particle size, solubility, deposition, and disposition in the alveoli, percentage of particles cleared from the lung and ingested, and reduction or oxidation of the chromium species. Workers in chromium plants may also have ingested water contaminated by chromium. Assessment of cancer risks for sites other than the lung are further

limited by the generally small sample size of occupational cohorts, and the small number of expected cases at sites other than the lung. Small numbers of expected site-specific cancers thus makes interpretation of individual studies of little value. This situation has encouraged meta-analyses of occupational cohort studies, with the attendant assumptions and decisions necessary in that process. Meta-analyses of cancer incidence at sites other than the lung are further limited by wide differences in heterogeneity in the study designs, reported data, and potential biases (Paddle, 1997). The small number of expected cases at specific sites (e.g. stomach) remains a problem even for meta-analyses. Determination of a consistent outcome measure for GI cancer is another limitation. Individual studies may include or exclude different cancers (e.g. stomach, colon, rectum, liver, and pancreas). In any case, dose estimates for ingested chromium are not possible in the occupational populations. Paddle's review of 14 occupational cohorts exposed to chromium that had reported on gastrointestinal cancer concluded that the data did not meet reasonable criteria for justification of a meta-analysis. He specifically noted that there was marked heterogeneity of the exposure conditions and outcome measures, with inadequate control of potential confounding.

Taken together the occupational cohort studies of chromium do not provide support for an increased risk of gastric or gastrointestinal cancer. This conclusion has been reached by several agencies that have reviewed the data. IARC concluded in their 1990 *Monograph for Chromium, Nickel and Welding*, that "for cancers other than the lung and sino-nasal cavity no consistent pattern of cancer risk has been shown among workers exposed to chromium compounds." (IARC, 1990). IARC reported that 20 of the 26 studies looked at cancer outside of the respiratory system, and 13 reported relative risk estimates for stomach or digestive system cancer. Four of these 13 study populations cited in the IARC document had estimated relative risks for digestive-system cancers of one or less (Hayes et al. 1979; Korallus et al. 1982; Satoh et al. 1981; Watanabe and Fukuchi 1984). Eight had estimated relative risks for digestive system cancers greater than 1, but the risk estimates are not statistically significant at the 95% confidence level (Enterline 1974; Hayes et al. 1989; [Langard 1990; Langard et al. 1990; Langard and Norseth 1975, 1979; Langard and Vigander, 1983], Machle and Gregorius 1948; Royle 1975a,b; Sheffet et al. 1982; Silverstein et al. 1981; Sorahan et al. 1987; Taylor 1966). Only one (Pokrovskaya and Shabaynina 1973) had a significantly increased rate of a digestive system cancer (esophageal cancer) (IARC 1990).

Several epidemiological studies of chromate production workers have been published since the IARC 1990 Monograph. Some of these studies (Boice et al., 1999; Davies et al., 1991; Gibb et al., 2000; Korallus et al., 1993) offer further valuable evidence, because they generally employ the most current epidemiological methods, evaluate larger cohorts of workers, and have long follow-up periods. These studies generally show no increase in risk of stomach or gastrointestinal cancer, or increases that do not meet the generally accepted criteria for causality (e.g. dose-response). These studies are not reviewed in detail here.

The U.S. EPA's 1998 Toxicological Review of Hexavalent Chromium concluded, "At present, the carcinogenicity of hexavalent chromium by the oral route of exposure cannot be determined because of a lack of sufficient epidemiological or toxicological data." ATSDR similarly concluded that there was no support for chromium ingestion causing cancer of the gastrointestinal tract. (ATSDR, 1993, 1998, 1999).

Susceptible Populations

Particular concerns have been raised about two potential susceptible populations with regard to Cr(VI) in drinking water, infants and adults who do not secrete normal amounts of stomach acid due to genetic factors or excessive use of antacids. With regard to the risk to infants, the scientific literature suggests that the secretion of acid in the stomach of infants starts very early in life, achieving a stomach pH of about 3 within 24 hours of birth (Avery et al., 1966). Thus, normal infants probably should not be viewed as a particularly susceptible population based upon their having a stomach pH too high (alkaline) to cause reduction of ingested Cr(VI) to Cr(III). While eating a meal transiently raises the pH of the stomach, post-prandial pH elevation persists for only a few hours in children (Nagita et al., 1996) or adults (DeFlora et al., 1987), and there are usually chemical agents (antioxidants) in food that can reduce Cr(VI) to Cr(III). As far as adults taking antacids to neutralize "excess" stomach acid, the effects of antacids are transient, and many of the most popular over-the-counter agents result in a maximum stomach pH of about 5 (Kararli, 1995). This is about the pH of tea or coffee, and is still acidic enough to rapidly reduce Cr(VI) to Cr(III), according to in vitro rate studies by Kerger et al. (1996). Thus, we may conclude that the only true susceptible population in this context would be individuals with genetic defects that preclude synthesis and secretion of stomach acid; these are, fortunately, very rare individuals.

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CHAPTER 5: OCCURRENCE OF CHROMIUM(VI) IN DRINKING WATER

Environmental Occurrence of Chromium

Chromium is a relatively abundant trace element, with an average concentration of 185 ppm in the continental crust (Taylor and McLennan, 1985). The highest grades of chromium ore, chromite, contain 52-56% chromic oxide, Cr_2O_3 (National Research Council, 1974). Less enriched, but still high concentrations of chromium are associated with the American Cordillarian orogenic belt that stretches meridonally from the Aleutians through California to Tierra del Fuego, including the Franciscan thrust complex containing small chromite deposits in California (Stowe, 1987a).

As a consequence, relatively large concentrations of chromium are present in geological formations throughout California. These deposits are commonly found in the western slopes of the Sierra Nevada and the Coastal Ranges of California (Stowe, 1987b). These include 25 thousand tons (Ktons) of known chromium ores and another 75 Ktons of estimated chromium ores (Stowe, 1987b).

The natural distribution of chromium in the environment has been perturbed by emissions of industrial chromium. These include releases from the production and uses of chromium in paints, metal alloys, chrome plating, corrosion inhibitors, refractory bricks, printing inks, photographic film, wood preserving, leather tanning, as well as releases of chromium from coal combustion and applications of sewage sludge in landfills (National Research Council, 1974; Deutsch, 1997). The industrial emissions of chromium to the atmosphere (7,340-53,610 $x10^3$ Kg/yr), soil (484-1,309 x 10^6 Kg/yr), and water (45-239 x 10^6 Kg/yr) have been grossly quantified on a global scale (Nriagu and Pacyna, 1988). But, there has been no comparable estimate of the magnitude of chromium contamination in California.

Ratio of Cr(III) to Cr(VI) in Groundwater Samples

While chromium has oxidation states ranging from Cr^{-2} to Cr^{+6}, only two are commonly found in the environment: trivalent Cr^{+3} or Cr(III) and hexavalent Cr^{+6} or Cr(VI). The relative distribution of the two species is commonly illustrated with an Eh:pH diagram. It shows the thermodynamically dominant species as a function of the system's redox potential (Eh) and its hydrogen ion activity (pH).

The thermodynamically dominant distribution of inorganic chromium species in groundwater is shown in Figure 1 (Deutsch, 1997), along with the stability field for predominant precipitated phase, chromium hydroxide ($CrOH_3$), between pH 6 and pH 12 Rai et al., 1987). The diagram indicates that the stable redox state of chromium in most groundwater environments (pH 6.5 to 8.5, and reducing to slightly oxidizing) are Cr(III) species [$CrOH^{2+}$, $Cr(OH_2^{+}$, $Cr(OH)_3^{0}$), while the anionic Cr(VI) species [$HCrO^{4-}$, CrO_4^{2}] are dominant in more oxidizing conditions (Rai and Zachara, 1984). However, Cr(III) species are more particle reactive than Cr(VI) species, and Cr(VI) minerals are commonly more soluble than Cr(III) minerals at most aquifer Eh/pH ranges (Deutsch, 1997).

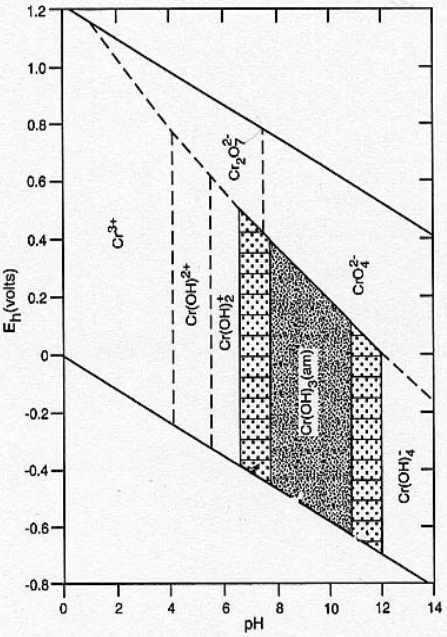

Figure 11-1 Chromium speciation and mineral equilibrium.

Figure 1. Chromium speciation and mineral equilibrium in groundwater (from Deutch, 1997)

However, the presence or absence of Cr(VI) in any groundwater system can not be assumed from simple thermodynamic calculations of what should be present at any given conditions of pH and Eh. The specific chemical forms of chromium present are influenced by the presence of other inorganic ions and minerals, dissolved and particulate organics, oxidants and reductants, and physical parameters (James and Bartlett, 1983; Loyux-Lawniczak et al., 2001). Moreover, the kinetics of some chromium redox reactions are relatively slow, so thermodynamic equilibrium may not be attained in some aqueous systems. Consequently, the amount of each chemical species of chromium present must be directly measured in all groundwater systems.

Analytical Issues and Concerns

Problems with accurate measurements of chromium in the environment have been recognized for decades. The National Academy of Sciences report (NRC, 1977) on the medical and biologic effects of chromium as an environmental pollutant stated:

"Adequate discussion of the distribution of chromium in the environment rests on the assumption that the data in the published literature are reliable. In the case of chromium and other metals, this assumption cannot be made-primarily because the analytical methods and sampling techniques used by investigators in the past have been, as a whole, unreliable and highly variable. The discrepancies in analytic data are particularly evident in literature discussions of chromium concentrations in the so-called trace range-i.e. in parts per billion. This is especially true of data on chromium concentrations in water, air, and biologic samples. For these reasons, it is difficult to assess properly the chromium concentrations reported in this chapter. In reviewing

the data, one should keep in mind that the best analytical methods were used, but we now know that those methods were not adequate, compared with recent refinements in analytic methodology."

The report then concluded with an Appendix on the analysis for chromium. It was prefaced with the following statement:

"The subject of sampling and analytic methods, of particular importance in dealing with chromium, contains many unknowns. The greatest potential error lies in the methods of sampling. The overall environmental program under consideration must be clearly defined before an experimental design is formulated and the investigation initiated. After the accumulation of appropriate field samples, regardless of the type of matrix involved, an analytic method must be appropriately selected on the basis of its capabilities with regard to precision, sensitivity, and accuracy. Chromium presents a serious problem, in that the analytic methods presently acceptable in terms of precision, sensitivity, and accuracy are expensive. Much headway has been made in the last 2 years in analytic development, but these efforts must be strengthened."

In spite of that recommendation, the adaptation of suitable analytical techniques for accurate measurements of chromium and other trace element concentrations in the environment were not quickly strengthened. This failure was extensively documented in subsequent reports delineating the failure of standard protocols to accurately measure trace element concentrations in aqueous systems (Flegal and Coale, 1989; Windom et al., 1991; Benoit, 1994). As a consequence of those criticisms, new methodologies were promulgated by state and federal agencies in the mid-1990s, including one for measuring hexavalent chromium [Cr(VI)] (USEPA, 1995). Still, the problem of accurately measuring environmental concentrations of chromium, and its inorganic species, has persisted.

This problem was recently demonstrated by a comparison of measurements of chromium in groundwater wells at a site in central California, which had reportedly been contaminated with chromium (Creasey and Flegal, 1999). That report was initially substantiated by analyses performed by a consulting firm using procedures consistent with standard industry practices for sampling and a California Department of Health Services-certified laboratory for analysis of EPA priority-pollutant metals in water. These studies reported chromium concentrations ranged from 62 to 163 parts-per-billion in groundwater at the contaminated site, and from and 17 to 51 parts-per-billion in groundwater at the adjacent site, during two sampling periods. In contrast, analyses using rigorous sampling and analytical techniques made in between those two periods actually determined the concentrations of chromium were 4 parts per billion in groundwater at the contaminated site and 0.07 parts-per-billion in groundwater at the adjacent site. These more rigorous results are from 15- to 700- times lower than chromium concentrations reported from DHS/EPA-certified protocols. Thus, the standard analyses performed by the methodology currently being used by regulatory agencies for monitoring of wells and groundwater in California indicated that chromium concentrations in both wells exceeded the California Maximum Concentration Level (MCL). However, more rigorous sampling and analysis methods demonstrated that chromium concentrations in both of those wells were far below that MCL (Figure 2).

As previously noted, the contrast between the two sets of measurements is attributed to their differences in both sampling and analytical techniques. Standard purging and pumping techniques can stress low flow aquifer formations and filter pack, increasing the colloidal and particulate loads of the samples well above their in situ levels. This has been shown by Puls et

al. (1992), who reported that low flow pumping rates (< 1 L/min) reduced the chromium concentrations measured in groundwater by a factor of two or more. Similarly, the use of standard sampling materials can introduce additional chromium to the sample and erroneously elevate the amount reported. Consequently, both sampling and analyses for trace concentrations of chromium require the use of acid-cleaned, non-metallic materials and trace metal clean techniques (e.g., acid cleaned containers, high purity reagents, and HEPA filtered laboratories), as initially detailed by Patterson and Settle (1976). While low flow pumping rates are needed for accurate measurements of chromium concentrations in groundwater monitoring wells, they are not needed for accurate measurements of chromium concentrations in water delivery systems where samples are taken directly from the pipelines. However, trace metal clean techniques are needed for accurate measurements of chromium concentrations in both groundwater-monitoring wells and in water delivery systems.

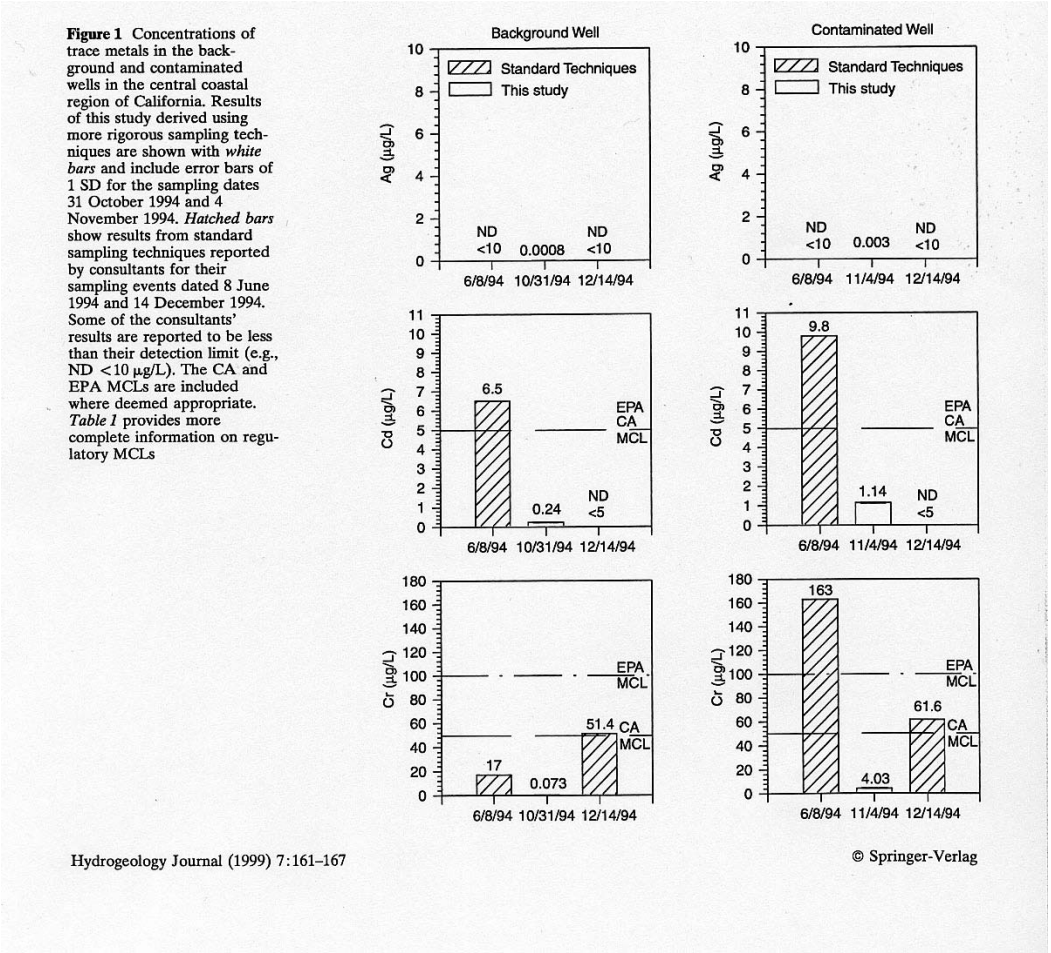

Figure 1 Concentrations of trace metals in the background and contaminated wells in the central coastal region of California. Results of this study derived using more rigorous sampling techniques are shown with *white bars* and include error bars of 1 SD for the sampling dates 31 October 1994 and 4 November 1994. *Hatched bars* show results from standard sampling techniques reported by consultants for their sampling events dated 8 June 1994 and 14 December 1994. Some of the consultants' results are reported to be less than their detection limit (e.g., ND <10 µg/L). The CA and EPA MCLs are included where deemed appropriate. *Table 1* provides more complete information on regulatory MCLs

Figure 2 (from Creasey and Flegal, 1999)

Conclusions

Chromium concentrations in groundwater may be elevated by both natural and anthropogenic processes. Chromium can and will occur naturally as a contaminant in some California water supplies through natural processes. The occurrence of specific chromium species in groundwater is highly variable and each source must be measured directly. Current data being reported from local and state monitoring agencies on ratios of Cr(VI): Cr(III) in specific sources of water include many sources with much higher ratios than were assumed for the OEHHA risk assessment for Cr(VI). These reports have been the source of a great deal of

concern and alarm, especially in Southern California. Accurate and reliable information on Cr(VI) content of drinking water supplies in California is a critical need that may not be being appropriately addressed currently.

Reported measurements of chromium concentrations and speciation in California groundwater using the EPA standard method are suspect, and may be giving spuriously high values. Further work by laboratories with experience and expertise in trace metal clean techniques to validate sampling and analysis methods for trace levels of both total dissolved chromium and Cr(VI) should be initiated immediately. This problem may be more appropriately addressed in academic laboratories than by regulatory agencies or environmental consulting firms, which may be deficient in this specific type of expertise.

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APPENDIX I:

Charge to the Panel

The Committee is to present written recommendations, and their scientific basis, to the Director of OEHHA, on the questions below regarding the potential carcinogenic risks of chromium(VI) in drinking water, based on an evaluation of the scientific literature and exclusively on public health considerations as described in the introduction. OEHHA will consider these recommendations in developing a Cr(VI) PHG.

The Evaluation will Focus on the Following Questions

1) Considering the toxicology, epidemiology and mechanistic information available regarding Cr(VI), should Cr(VI) be considered as posing a carcinogenic risk by the oral route?

2) If Cr(VI) is to be considered as posing a carcinogenic risk by the oral route, what approaches does the Committee suggest to establish a PHG?
   - The Borneff et al., (1968) mouse study is the only animal drinking water study of which we are aware that was designed to look at the potential carcinogenic effects of Cr(VI). We are seeking your comments on the strengths and weaknesses of this study for purposes of making a quantitative estimate of the cancer risk for Cr(VI) in humans.
   - Does the epidemiology literature contain studies that would be useful to derive a cancer potency for Cr(VI), such as, using the occupational data reporting excess gastrointestinal or other non-respiratory tumors?
   - If the available literature does not allow the development of a cancer potency factor, what studies are available that would allow the development of a PHG that could take into account potential cancer risks from Cr(VI). For example, some agencies apply an additional safety factor to the non-cancer chronic health effects observed in animals.

3) The conversion of Cr(VI) to Cr(III) by simple chemical reactions in the stomach and pharmacokinetics after absorption can influence the toxic effects of Cr(VI).
   - How can the effects be quantified and the results applied as part of the approach in developing a PHG? As examples:
     (1) Change the slope of the dose-response curve;
     (2) Change the shape of the dose-response curve.
   - Is there literature that indicates variability in the general population in the conversion of Cr(VI) to Cr(III)?
   - Is there adequate information to identify a threshold for the oral route of exposure?