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October 19, 2009

Comments to the draft document entitled "Public Health Goal for Hexavalent Chromium in Drinking Water", prepared by the California Environmental Protection Agency (Office of Environmental Health Hazard Assessment)

Although I find that several parts of the OEHHA Document are written appropriately and with competence, I have some important reasons of concern, as follows.

Comment #1. Detoxification of Cr(VI) in the organism

I started working on this subject more than 30 years ago, by investigating Cr(VI) reduction in blood and liver (e.g., S. De Flora, *Nature* 271, 455-6, 1978; F.L. Petrilli and S. De Flora, *Mutat. Res.* 54, 139-147, 1978), in the respiratory tract (e.g., F.L. Petrilli *et al.*, *J. Clin. Invest.* 77, 1917-24, 1986; S. De Flora *et al.*, *Cancer Res.* 47, 4740-5, 1987), in the gastric environment (e.g., S. De Flora *et al.*, *Mutat. Res.* 192, 159-174, 1987), and in other body compartments (reviewed in S. De Flora *et al.*, *Carcinogenesis*, 18, 531-7, 1997). Some of my papers are correctly reported in the OEHHA Document.

These data, generated in *ex vivo* studies in both humans and animal models, led me to develop the theory that Cr(VI) genotoxicity and potential carcinogenicity tend to be attenuated or suppressed in the body. This theory is widely accepted in the international literature. For instance, when commenting my data, the IARC Working Group "interpreted these findings as indicating mechanisms that limit the activity of Cr(VI) compounds *in vivo*" (IARC Monographs, Vol. 49, 1990). In the ATSDR Document, the US Department of Health and Human Services indicated that these "mechanisms limit the bioavailability and attenuate the potential effects of Cr(VI) compounds *in vivo*. Thus, the oral toxicity of chromium is low" (US Dept. of Health and Human Sciences, 1993). The USEPA concluded that "the body's normal physiology provides detoxification for Cr(VI)" (USEPA, 1991).

Although it is evident that Cr(VI) detoxification mechanisms represent formidable barriers against Cr(VI) toxicity, genotoxicity, and carcinogenicity, I do not pretend that they are infinite and cannot be saturated. Under certain conditions, especially in animal models, they may be overwhelmed as a function of the dose and of the administration route (see Comment #9). Therefore, the statement, reported on page 17 of the OEHHA Document, that according to my studies the Cr(VI) detoxifying mechanisms in the organism are "essentially inhexhaustive" does neither reflect my opinion nor what is written in my papers.

Comment #2. Genotoxicity of oral Cr(VI) in the intestinal tract

On page 37 is stated that no study to date has looked for DNA damage in the oral cavity or gastrointestinal tract following oral administration of Cr(VI). It is also stated that these studies are needed. The authors of the document overlooked our *ad hoc* study (S. De Flora *et al.*, *Mutat. Res.* 659, 60-69, 2008), in which we demonstrated that the daily administration of sodium dichromate to SKH-1 mice, at the doses of 5 or 20 mg/L for 9 consecutive months, failed to enhance the frequency of DNA-protein crosslinks and did not cause oxidative DNA damage, measured in terms of 8-oxo-dGuo, in mouse forestomach, glandular stomach, and duodenum.

Comment #3. Genotoxicity studies of oral Cr(VI) in cells outside the GI tract

Table 2 and pages 37-41 of the OEHHA Document summarize studies on the genotoxicity of Cr(VI) administered by the oral route.

Again, relevant literature data were overlooked. In a study of mine (S. De Flora *et al.*, *Mutat. Res.* 610, 38-47, 2006), potassium dichromate and sodium dichromate failed to affect the frequency of micronucleated erythrocytes in bone marrow and peripheral blood of BDF1 and Swiss mice of both genders when administered with the drinking water, up to a concentration of as much as 500 mg Cr(VI)/L for up to 210 consecutive days. Even a single intragastric dose of 17.7 mg/kg body weight was negative. In addition, the same Cr(VI) salts, administered to pregnant Swiss albino mice, up to a concentration of 10 mg/L drinking water, did not cause any toxic or genotoxic effect in fetus liver or peripheral blood.

Surprisingly, unless I missed them somewhere else in the document, even the NTP studies evaluating the frequency of micronucleated erythrocytes in peripheral blood were not cited. These

studies (J. Bucher, *Toxic. Rep. Ser.* 72, 1-G4, 2007) showed that sodium dichromate was negative in both male and female B6C3F1 mice, in which the compound was administered for 3 consecutive months at concentrations ranging between 21.8 and 349.0 mg Cr(VI)/L. Sodium dichromate was also negative in male BALB/c mice, and gave results which were classified as equivocal in a further experiment in male B6C3F1 mice, in which the increase of micronucleated erythrocytes frequency did not reach the statistical significance threshold. In male C57BL/6 mice transgenic for *PhiX174am3*, the compound was positive in one experiment but negative in another one.

Thus, on the whole, when introduced with the drinking water at doses exceeding up to 10,000 times drinking water standards for total chromium, or even following massive intragastric administration, the bulk of evidence is that Cr(VI) compounds do not increase the frequency of micronucleated erythrocytes in mice of both genders and various age, belonging to a variety of strains (BDF1, Swiss albino, Swiss Webster, B6C3F1, and BALB/c).

Comment #4. The NTP carcinogenicity study in mice and rats

I believe that the NTP carcinogenicity study with sodium dichromate dihydrate was quite important, timely, and well executed. What I disagree with is the interpretation of the results obtained.

When I had the opportunity, years ago, to see the design of this study, I expected that forestomach tumors and perhaps glandular stomach would have developed at the highest doses of sodium dichromate tested, which were extremely high. The reason for this expectation is that, as specified in Comment #1, the detoxifying capacity of the gastric environment is not infinite. Moreover, as also explained in the OEHHA Document, the rodent forestomach is a vulnerable tissue, particularly to irritants such as high-dose Cr(VI). Indeed, evaluation of Cr(VI) carcinogenicity in the stomach was the major focus of the NTP study and the major premise for performance of this study. Therefore, it is particularly important that, even at the extremely high Cr(VI) doses tested, no stomach tumor was induced by sodium dichromate in either rodent species.

The conclusion of the NTP study, as reported in the only paper published in a peer-reviewed journal (Stout *et al.*, *Environ. Hlth Perspect.*, 117, 716-22, 2009), was that "Cr(VI) exposure resulted in increased incidences of rare neoplasms of the squamous epithelium that lines the oral cavity (oral mucosa and tongue) in male and female rats, and of the epithelium lining the small intestine in male and female mice". As noted in the OEHHA Document, a statistically significant increase of oral cancers only occurred at the highest dose tested (516 mg/L sodium dichromate) in both male and

female rats. A statistically significant increase of small intestine tumors only occurred at the highest dose tested in male mice (257.4 mg/L) and at the two highest doses tested in female mice (172 and 516 mg/l). These are huge doses! One should go to the lab and see the color and appearance of water containing hundreds or even tens mg/L Cr(VI). Nobody would drink this water unless for suicidal purposes (which probably would be unsuccessful, see Comment #8). No effect was observed at the lowest doses tested in the NTP study, corresponding to 5-30 mg Cr(VI)/L water (which still are quite high doses), which is in agreement with the conclusions of our genotoxicity study (S. De Flora *et al*., *Mutat. Res.* 659, 60-67, 2008), ruling out that DNA damage may occur not only in the forestomach and glandular stomach but also in the duodenum of mice receiving sodium dichromate with the drinking water, at the doses of 5 and 20 mg Cr(VI)/L (see Comment #2).

It should be noted that in the NTP study there were significant decreases of certain tumors in Cr(VI)-treated rodents, such as a decrease of total benign tumors in both rats (females only) and mice (males only), which by the way was the only concomitant change in the two rodent species, a decrease of pituitary gland tumors in both male and female mice, and a decrease of liver adenomas in both male and female mice, which was the only effect observed at 2 or 3 Cr(VI) concentrations. Clearly, although these decreases are statistically significant, they do not mean that Cr(VI) is protective but highlight the fact that, likewise, significant increases at high doses are not biologically significant and do not bear relevance to the human situation.

Comment #5. Carcinogenic potency of Cr(VI) in humans

On page 58, last paragraph, it is stated that IARC (1990) concluded that Cr(VI) is a "strong" carcinogen for the respiratory system. This statement is not correct. As quoted on page 42 of the OEHHA Document, the IARC concluded that "there is sufficient evidence in humans for the carcinogenicity of Cr(VI) compounds as encountered in the chromate production, chromate pigment industry and chromium plating industries", i.e., only in 3 occupational settings (out of hundreds) that in the past involved the inhalation of very high Cr(VI) doses, often leading to ulcers and perforations of the nasal septum. The need for high Cr(VI) doses to induce lung cancer is confirmed by more recent study, such as the Gibb *et al.* (2000) study, which is extensively reported and discussed in the OEHHA Document. As everybody knows, the Mancuso's data, that U.S. EPA used for the potency estimate, are highly biased.

Comment #6. Carcinogenicity of Cr(VI) in animal models

Apart from the NTP study, this subject is discussed on pages 56-57, where it is concluded that "rodents are relatively insensitive to Cr(VI) when it is administered by inhalation". It may be added that the large majority of the studies reviewed in detail by IARC (see pages 115-142 of the Monograph) were negative, and most positive data were generated at implant sites only and at a single, high doses, i.e., under conditions that could by-pass or overwhelm the body defense mechanisms.

Comment #7. Link between inhalation exposure to Cr(VI) and cancer of digestive organs

In 1988, the WHO concluded that "there is insufficient evidence to implicate chromium as a causative agent of cancer in any organ other than the lung" (WHO, *Environmental Health Criteria*, Vol. 61, 1988). In 1990, the IARC concluded that "for cancers other than of the lung and sinonasal cavity, no consistent pattern of cancer risk has been shown among workers exposed to chromium compounds" (IARC Monograph, Vol. 49, 1990). This conclusion was reiterated, almost *verbatim*, in a review article published 3 years later (Cohen *et al.*, *Crit. Rev. Toxicol.* 23, 255-81, 1993). My further analysis of recent studies confirmed these conclusions (S. De Flora, *Carcinogenesis*, 21, 533-541, 2000).

In the last paragraph of page 72, the OEHHA Document concludes that "a summary of the findings of multiple studies where workers were exposed to Cr(VI) by the inhalation route (conducted by OEHHA) was suggestive of a link between inhalation exposure to Cr(VI) and cancer of the digestive organs". This conclusion is surprising and contrasts with the actual results of the OEHHA study, which are reported in Tables 7 and 8 on pages 62-69. In fact, taking into account statistically significant variations, the analysis of 30 studies led to the following results for cancers of the digestive system:

– Oral cavity and pharynx: no significant change in 9 studies. *Note that the results in humans do not agree with the results of the NTP carcinogenicity study in the oral cavity of mice*.
– All digestive: significant increase in 2/10 (20%).
– Esophagus: no significant change in 10 studies.
– Stomach: significant increase in 3/25 (12%). Note that, at least in two studies, exposure to compounds other than Cr(VI) may have occurred.
– Colon: no significant increase in 16 studies. Interestingly, in 4 studies (Axelsson, 1980; Deschamps, 1995; Moulin, 1990; Sorahan, 2000) the cancer data for colon also included data for cancer of the small intestine. *Again, note that the results in humans do not agree with the results of the NTP carcinogenicity study in the small intestine of rats.*

– Rectum: no significant change in 11 studies.
– Liver and gall bladder: no significant increase in 9 studies and, on the contrary, a significant decrease in 1 study (11.1%).
– Pancreas: no significant change in 12 studies.

The analysis of the same epidemiological studies led to the following results for tumors of the respiratory tract:
– Lung cancer: significant increase in 18/29 studies (62.1%).
– Nonmalignant respiratory diseases: no increase in 18 studies but, on the contrary, a significant decreases in 4 (22.2%) studies (Birk, 2006; Hayes, 1989: Korallus, 1993; Moulin, 1990).

It is well known that, to reach a conclusion, epidemiological data have to be consistent in different studies, a requirement that was taken into account by the IARC Working Group. In this light, the conclusion of the OEHHA Document does not appear to be supported by the results of the OEHHA study.

Comment #8. Examination of evidence for chromium carcinogenicity

This Section of the OEHHA Document (pages 72-74) tries to summarize the data reported in the previous pages. In my comment, I will follow the same subtitles used in the Document.

Human studies. In addition to the considerations on the carcinogenic potency (see Comment #5) and on the link between inhalation exposure to Cr(VI) and cancer of digestive organs (see Comment #7), the OEHHA Document relies on the Chinese study, whose limitations are extensively discussed on pages 69-71. Note that this controversial study was further examined in a recent article (B.D. Kerger *et al.*, *J. Toxicol. Environ. Hlth*, 72, 329-44, 2009), which is not quoted in the Document.

Animal studies. I already forwarded my considerations on the interpretation of the NTP study (see Comment #4). As to the Borneff *et al.* (1968) study, which is extensively reported and discussed both in the text and in Appendix D of the OEHHA Document, this study was so obsolete, inadequate and full of problems that the IARC Working Group (including myself and other 20 scientists) decided not even to cite it in the 1990 Monograph. Incidentally, it is noteworthy that the Borneff *et al.*, study suggested an increase of forestomach tumors in mice (that even the author

interpreted with a great caution) while the NTP study suggested an increase of small intestine tumors in mice. Who is right?

Genotoxicity. As previously discussed (Comments #2 and #3), the data reported in the OEHHA Document are largely incomplete.

Toxicokinetics. As previously discussed (Comment #1), I do not pretend that detoxification mechanisms are infinite. In any case, they are formidable barriers that imprint a threshold character to Cr(VI) carcinogenesis (see Comment #9).

Toxicity. It should be noted that the oral toxicity of Cr(VI) is rather low. In rodents, the LD_{50} by the oral route is >50 mg Cr(VI)/kg body weight (S.C. Gad *et al.*, 1986, which is cited elsewhere in the Document). Furthermore, the NTP carcinogenicity study provided evidence for the great tolerability of high-dose Cr(VI). In humans, the OEHHA Document provides on page 57 two examples of fatal acute ingestion of Cr(VI), in both cases at doses of hundreds milligrams. There are several other reports of episodes of accidental ingestion or tentatives of suicides with Cr(VI), most of which luckily failed. For instance, following ingestion of Cr(VI) compounds in the 8-20 g range, 8 subjects survived and 3 subjects died (S. De Flora *et al.*, in Berthon, G. (ed.) *Handbook of Metal Ligand Interactions in Biological Fluids. Bioinorganic Medicine*, Marcel Dekker, NY, Vol. 2, pp. 716-25).

Mechanism. This section of the Document summarizes some mechanisms of Cr(VI). Regarding the meaning of the intracellular Cr(VI) reduction, when in 1989 I prepared a review (cited in the Document) together with the late Karen Wetterhahn, the best researcher on Cr(VI) biochemical toxicology ever, we agreed on the interpretation that when Cr(VI) reduction occurs close to DNA target molecules, it is an activation mechanism (uptake-activation theory). However, when Cr(VI) reduction occurs in the cell cytoplasm or in any case far away from DNA, it is a detoxification (uptake-detoxification theory), due to the myriad of intracellular ligands that block Cr(VI) or its derivatives before reacting with DNA. Here is a further mechanism responsible for the occurrence of thresholds in Cr(VI) toxicology.

Conclusion. It is surprising that this chapter reaches the conclusion that "the findings of available human, animal, genotoxic, and toxicokinetics studies all indicate that Cr(VI) is a possible human carcinogen by the oral route". It is intriguing that all data that were evaluated to be either

incomplete or heavily criticized in the Document itself now become the starting point to reach the above conclusion and to develop a proposal of PHG.

Comment #9. Thresholds

I started developing the firm belief that the carcinogenicity of certain compounds is governed by threshold mechanisms in 1978. In that year I published a study in which I demonstrated that genotoxic compounds that tend to be detoxified in the body, among which sodium dichromate, are either not carcinogenic or carcinogenic only at certain conditions (S. De Flora, *Nature*, 271, 455-6, 1978). Thereafter, I validated this theory in a number of studies. Some of my papers have the keyword "threshold" in the title (e.g., S. De Flora, *Toxicol. Pathol.*, 12, 337-43, 1984; S. De Flora, *Ann. Am. Conf. Ind. Hyg.*, 12, 145-55, 1988; F.L. Petrilli and S. De Flora, *Sci. Total Environ.*, 71, 357-64, 1988; S. De Flora, *Carcinogenesis*, 21, 533-41, 2000) or in subtitles (e.g., S. De Flora and K. Wetterhahn, *Life Chem. Rep.*, 7, 169-244, 1989).

All the patterns that characterize Cr(VI) toxicity and genotoxicity *in vivo* and carcinogenicity in both humans and animal models, along with mechanistic considerations, as previously discussed, point to the existence of threshold mechanisms in Cr(VI) toxicity and carcinogenicity. As previously mentioned (Comment #1), the existence of thresholds for Cr(VI) is widely accepted in the international literature and by major Agencies, such as IARC, U.S. EPA, and U.S. Dept. of Health and Human Sciences.

The lack of thresholds, as claimed in APPENDIX A of the OEHHA Document, would imply that even a single Cr(VI) molecule, introduced in the organism, would be able to reach the DNA of target cells, which is unbelievable. It should be added that threshold mechanisms occur not only at toxicokinetic and metabolic levels but also after DNA damage, e.g., due to DNA repair and apoptosis. My lab investigated these processes by analyzing *in vivo* both transcriptome (A. Izzotti *et al.*, *Mol. Carcinogenesis*, 35, 75-84, 2002) and proteome (A. Izzotti *et al.*, *Int. J. Oncol.*, 24, 1513-22, 2004).

Comment #10. Potency calculation and evaluation of PHG

Having worked for 49 years in an Institute of Hygiene and Preventive Medicine and a Department of Health Sciences at the University, I appreciate any effort to guard public health. However, starting from inconsistent epidemiological and experimental data and denying the occurrence of threshold mechanisms in Cr(VI) toxicity and carcinogenicity lead to unrealistic figures.

The proposed PHG for Cr(VI) of 0.06 ppb (µg/L) in drinking water means that it is believed that concentrations higher than that, of course with a safety margin, are not detoxified in the body after oral intake. The results of the NTP carcinogenicity study in mice and rats that, as noted in Comment #7, were not consistent with the results of epidemiological studies, were used as a major conceptual base for claiming that Cr(VI) is carcinogenic also by the oral route and for calculating the proposed PHG. The concentrations of Cr(VI) in water that produced significant variations of tumor incidence in the NTP study were in the range of hundreds mg/L, i.e., millions of times higher than the proposed 0.06 µg/L PHG.