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Re: Comments on Peer Review Draft of NTP TR 546, Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate in F344/N Rats and B6C3F1 Mice (Issued in April, 2007)

Dear Dr. Shane:

We are submitting comments on the draft NTP TR 546 report on sodium dichromate dihydrate. Understanding the potential hazards of hexavalent chromium compounds is an important public health consideration.

The dichromate study performed by NTP must be interpreted in light of the extensive available scientific literature on chromium compounds if one wishes to predict the human health hazards at doses lower than those tested by NTP. These comments are intended to help NTP in drafting their next document which might discuss the significance of this study with respect to public health.

Our key comments on the draft report can be summarized as follows:

1. The practical MTD was exceeded. The test conditions almost certainly exceeded any practical maximum tolerated dose (MTD), since there were severe reductions in water intake and probable impacts on hydration and nutrition that affected tumor rates in the highest dose groups.

The significantly decreased water intake at high dichromate concentrations appears to stem from poor palatability, which caused weight loss and avoidance of water intake at high doses (that did not occur at low doses). There is evidence suggesting that this palatability-related effect altered the tumor response at high doses and that, accordingly, the results are not applicable to predicting the human response following chronic exposure to plausible concentrations (i.e., below 1 mg/L).

The mouse small intestine tumorigenicity is likely an artifact of exceeding the MTD in this strain/species. The species-specific occurrence of small intestine tumors in mice and the coinciding cellular changes in the small intestine, pancreas and liver are indicative of a response (small intestine

tumors) that is better characterized as an uncertain finding likely due to multiple factors not present at lower doses. Only one other substance tested by NTP (captan feeding study) has elicited a similar response in the B6C3F1 mouse, and this finding was subsequently considered by NTP and others to be an exclusively high-dose phenomenon. In 2004, EPA reclassified captan from status as a B2 carcinogen to being “not likely to be a human carcinogen at dose levels that do not cause cytotoxicity and hyperplasia” and that “the latter is a key event in the sequential cascade of events leading to cancer” (Federal Register, 2004). We believe that the observed intestinal tumors from captan and from dichromate may be due primarily to chronic diminution of hydration and nutrition in B6C3F1 mice which occurs only at doses that exceed the practical MTD for this species.

2. The role of chemical reduction and chromium toxicokinetics did not receive adequate discussion or consideration. The discussion section of the draft report did not adequately consider and objectively discuss the scientific literature which has shown that gastrointestinal tract toxicity following chronic oral exposure is limited by the chemical reduction of dichromate to Cr III by natural fluid secretions, foods, and tissues (a type of protective mechanism which is most relevant to potential long-term drinking water exposures in human populations).

The literature which NTP chose to discuss on this topic seems to have been selected to support a conclusion that oral detoxification mechanisms for dichromate may not be relevant to humans. We suggest that NTP re-examine the historical discussions on this issue by IARC and other agencies, as well as several more recent research papers we discuss below. For example, we have published several relevant research papers on this topic (most of which were not cited in the NTP report).

We believe that the “Discussion” section in this particular NTP report goes far beyond the typical discussion offered by NTP when they discuss the findings and implications of their animal studies. If NTP chooses to evaluate the epidemiological and human health risk assessment aspects of oral dichromate exposures in this bioassay report, we recommend that the discussion be revised to reflect a more objective, balanced, and reasonably comprehensive assessment of the available scientific literature.

3. The current “Discussion” section should highlight the critical finding of no treatment-related tumors outside the gastrointestinal tract. Animal studies over the past 5 decades have demonstrated that dichromate is a ‘portal of entry’ or contact tumorigen, with no tumorigenic response beyond the site of entry. The NTP bioassay findings also support this conclusion.

The oral tumors observed in the dichromate-treated rats demonstrate that very high chronic exposures result in portal of entry tumorigenesis. The

lack of treatment-related tumors beyond this site in the rat is further supportive of prior animal studies demonstrating a lack of response in organs guarded by body fluids or tissues with appreciable reduction capacity to detoxify low to moderate doses of dichromate. The absence of oral, forestomach, or glandular stomach tumors in mice is also consistent with the known oral detoxification mechanisms for dichromate. However, the unusual occurrence of mouse small intestine tumors at the highest doses is an unexplained inconsistency that deserves further study given its isolated occurrence in the NTP studies and its lack of coherence with the existing literature on Cr(VI) toxicokinetics and mode of action.

Each of the above points is discussed in more detail in the numbered paragraphs below.

Maximum Tolerated Doses. The 90-day and 2-year NTP study findings on dichromate suggest that biologically important reductions in water intake and food intake led to substantive reductions in body weight at the two highest doses in rats and mice of each species. While the body weight reductions might be within acceptable guidelines for MTD criteria, water intake was quite clearly and significantly impacted and should be considered in the MTD determination. We suspect that overt impacts on hydration and nutrition influenced these highest dose findings (i.e., mouse small intestine hyperplasia and tumors) in a manner that cannot be reasonably extrapolated to lower dose conditions, especially in humans with plausible chronic oral exposures below 1 mg/L. Our own research in humans has demonstrated humans can readily reduce up to 10 mg Cr(VI)/L concentrations of ingested dichromate to Cr(III) without exhibiting toxicity or elevated biomarkers for systemic uptake as Cr(VI) (see Finley et al, 1997; Kerger et al, 1996a; Paustenbach et al, 1996; Kuykendall et al., 1996; Kerger et al., 1997; De Flora et al., 1997; Corbett et al., 1998; reviewed in Paustenbach et al., 2003).

In concert with body weight reductions in the dichromate 2-year bioassay, there were quite substantial reductions in average weekly water consumption relative to control intake for rats and mice of both sexes. Examples include that for male mice at 257.4 mg/l (22 to 43% reduced) and 85.7 mg/L (0 to 20% reduced), and similarly for female mice at 516 mg/L (20 to 50% reduced), 172 mg/L (0 to 31% reduced), and 85.7 mg/L (0 to 23% reduced). We believe the combined impact of lowered hydration and lowered body weight/nutrition in the high dose animals deserves further consideration in regards to defining the MTD, since these outcomes apparently influenced the tumor rates as discussed further below.

The 90-day toxicity study by NTP indicated that higher doses in mice were associated with lowered body weight that was apparently due to reduced water consumption rather than due to dichromate toxicity (NTP TR-72, page 59). This raises the question as to whether or not the chronic health consequences of poor palatability should be considered a “toxic response” due to dichromate, i.e., specifically in relation to the unusual small intestine tumor response in mice.

Reduced hydration could adversely impact the process of normal digestion in these high dose mice with respect to normal bulk flow. Chronic low hydration may significantly alter biliary and/or pancreatic secretions required to properly wet and digest food in a manner independent of any Cr(VI) effect. The lowered hydration appears well correlated with pancreatic acinar cell granule depletion, a finding suggestive of overtaxed pancreatic enzyme secretions which were not observed at doses where normal water intake occurred. The lowered hydration also appears to be correlated with the elevated occurrence of the primarily singleton tumors in the small intestines of the highest dose mice.

Based on the available data, the combination of lowered hydration, nutrition, and body weight directly or indirectly influenced the outcome of this bioassay and may have been a consequence of poor palatability rather than being a specific response to Cr(VI) toxicity. There were liver changes suggestive of glycogen depletion in mice at the highest doses in the 2-year study which could be explained by reduced nutritional status in these groups. Nutrition and obesity have been documented over the past 4 decades to impact the frequency and distribution of tumors observed in control mice and rats (Rowlatt et al., 1973; Clayson, 1975; Tucker, 1979; Lagopoulos and Stalder, 1987; Lagopoulos et al., 1991; Fu et al., 1994; James and Muskhelishvili, 1994; Kolaja et al., 1996; NTP, 1997b; Yoshida et al., 1999; Busch et al., 2004; Spindler, 2005). Rodent caloric restriction reduces mammary and pituitary tumors in rats and liver tumors in mice. The dichromate bioassay demonstrated these species-specific tumor reductions at the highest doses where poor palatability led to lowered hydration, nutrition, and body weight.

Mouse Small Intestine Tumor Response. As noted above, we have some serious concerns about how to interpret the occurrence of mouse small intestine tumors. The predominantly solitary tumors of the duodenum or jejunum apparently occurred only in those mice with severely lowered water intake and consequently lowered body weight/nutrition. The available data on non-neoplastic lesions of the small intestine of dichromate-treated mice do not indicate a pattern of diffuse irritation, ulceration and multiple tumors that one might reasonably expect from repeated direct contact with sufficiently high concentrations of a chemical oxidant dispersed in a food bolus. It seems likely that the chemical reduction capacity of gastrointestinal secretions was overwhelmed at the highest doses in both mice and rats, but this unusual, isolated response in the mouse deserves further study.

While the small intestine tumors represent a clear tumorigenic response at the highest dichromate doses in mice, there is a lack of cogent discussion on the fact that this site (small intestine) was not a primary target organ for irritation, ulceration, or tumors from dichromate in prior rodent/animal studies, including the 90-day studies by NTP in rats and 3 different species of mice. Likewise, there has never been an association of hexavalent chromium exposure and small intestine tumors in humans. We believe that the discussion should note the absence of small intestine tumors in prior chronic studies of oral dichromate exposure in humans (reviewed in IARC, 1990; ATSDR, 2000; De Flora, 2000) and other mammalian species (Mackenzie et al., 1958; NTP, 1996a, 1996b, 1997; Grosse and Heller, 1946; Anwar et al., 1961; Maruyama et al., 1982; Borneff et al.,

1968). NTP should more carefully consider the apparently correlated factors of overtaxing of gastrointestinal secretions in the face of reduced hydration and nutrition at the highest doses in mice and rats. The apparently isolated and multi-factorial nature of the small intestine tumors in mice makes these findings of uncertain relevance to humans. The absence of such conditions and responses at dichromate concentrations more relevant to plausible chronic exposures in humans (i.e., below 1 mg Cr(VI)/L) is an important consideration that limits the extension of the NTP findings for human health risk assessment.

The available scientific literature and mechanistic information, including the findings from the high dose NTP 90-day studies in rats and mice, indicate that the oral cavity and forestomach of rodents, and secondarily the glandular stomach, would be the expected 'portal of entry' target organs for hexavalent chromium ingestion. We suggest that the NTP report discuss anatomical and physiological differences between the digestive tract tissues and secretory organs in mice versus rats because we expect that this might shed some light on the different histopathological responses observed between species. For example, differences in the reduction capacity of daily gastric juice secretions between species may be important.

In rats, the expected dichromate toxicity pattern at high doses seems to occur, with the expected tumorigenic response in the oral cavity and in the forestomach (based on the 90-day study) following chronic, diffuse inflammation and/or ulcerations at the site of contact. In mice, previous studies have indicated forestomach ulcers and tumors from ingestion exposures, but the 2-year study findings suggest that mice may be more resistant to both oral cavity and forestomach or glandular stomach lesions from hexavalent chromium than rats. The occurrence of small intestine tumors in the species that appears to be more resistant to ulcers and tumors in the organs that are most directly exposed and unprotected (by gastric juices) is better characterized as an uncertain finding in the current NTP 2-year study. As noted earlier, the other long-term studies in rats, mice, and dogs failed to identify the small intestine as a target organ at doses that can be tolerated for long-term oral exposures. This deserves special discussion in the revised NTP report.

The draft NTP report notes on page 84 that o-nitrotoluene (NTP, 2002) was one of two chemicals studied by NTP that induced an increase in "intestinal neoplasms" in mice. We find this to be somewhat misleading because the responses to this chemical were isolated to the large intestine, in contrast to the quite specific response localized to the duodenum and jejunum of the small intestine in the current study. In contrast to the dichromate study, o-nitrotoluene in the diet of mice also caused excess liver tumors and hemangiomas, and in the rat caused excess mesothelioma, skin, mammary, lung, and liver tumors.

Two other NTP bioassays reported excess large intestine tumors in rats but not in mice for bromodichloromethane (NTP, 1987) and 1-amino-2,4-dibromoanthraquinone (NTP, 1996d). Four NTP bioassays reported excess small intestine tumors in rats but not in mice for 2,2-bis(bromomethyl)-1,3-propanediol (NTP, 1996c), glycidol (NTP, 1990), 1,2,3-trichloropropane (NTP, 1993b), and 2,3-bromo-1-propanol (NTP, 1993a). Unlike the

dichromate study findings, these responses to other chemicals in the small or large intestines in rats were generally accompanied by multi-site tumorigenicity, often including multiple gastrointestinal tumor sites in both species.

The draft NTP report discussion on page 84 also mentions an older bioassay of dietary captan (NTP, 1977), which was associated with a relatively low incidence of duodenal tumors in B6C3F1 mice (up to 5/46 animals). The duodenal lesions were located approximately 1 cm posterior to the pylorus and included mucosal hyperplasia associated with an adenomatous polyp or polypoid carcinoma. The tested doses for captan, a water-insoluble pesticide known to cause diarrhea at high doses, was 8,000 or 16,000 ppm for 80 weeks. Rats did not exhibit duodenal tumors from captan, and mice did not exhibit any excess tumors outside the duodenum. The mean body weights of both low- and high-dose rats and high dose mice were distinctly lower than those of matched controls throughout the study. It seems plausible that the lowered body weight and associated lowered nutrition/hydration due to chronic diarrhea at the high dose of captan may have caused the unusual duodenal tumor response in B6C3F1 mice.

Although the mechanism(s) for the mouse small intestine tumor/hyperplasia response has not been fully elucidated, for captan it is clearly related to the precursor hyperplasia response that does not occur at – and is probably not relevant to – lower doses in the mice or at any dose in rats (Federal Register, 2004).

Two other NTP bioassays have identified excess small intestine tumors in male or female mice, but not in similarly exposed rats. This includes bioassays for 1,3-butadiene inhalation (NTP, 1993c) and for gavage administration of Direct Blue 218 (NTP, 1994). In both studies, the statistical excess of small intestine tumors predominantly among male mice was low incidence and not dose-related, leading to their inclusion under the category of “Uncertain Findings.” Unlike the dichromate bioassay, both 1,3-butadiene and Direct Blue 218 induced tumors at multiple sites in both mice and rats.

We strongly recommend that NTP consider these points and evaluate the possibility of conducting additional studies to understand the role of reduced water intake in the dichromate study results in regards to mouse small intestine tumors.

Portal of Entry Tumorigenesis. The findings of the NTP 2-year study are clearly in keeping with the existing literature on tumorigenic responses to hexavalent chromium in that oral exposure did not result in treatment-related tumors beyond the ‘portal of entry’, i.e., beyond the gastrointestinal tract. This point has not been clearly acknowledged in the discussion, and has instead been obscured by the incomplete literature review. Several chronic studies over the past 5 decades have suggested that mammalian exposures to hexavalent chromium via ingestion were not tumorigenic (Mackenzie et al., 1958; NTP, 1996a, 1996b, 1997; Grosse and Heller, 1946; Anwar et al., 1961; Maruyama, 1982; Borneff et al., 1968), in contrast to studies showing ‘portal of entry’ tumorigenesis following inhalation exposures (nasal and lung tumors) and direct injection or instillation of Cr(VI) (reviewed in IARC, 1990; ATSDR, 2000).

The draft NTP report contains an incomplete and unbalanced discussion of the available epidemiological, animal, and mechanistic studies (see pages 84-90) in a manner that seems to bolster a number of poorly supported hypotheses relating to oral chromium exposure and its possible implications in human populations. This type of discussion which attempts to blend epidemiology, case reports, acute toxicology, and plausible mechanism of action has never been offered in any prior NTP cancer bioassay report to the best of our knowledge.

For example, on page 84 it is stated that “Although lung cancer related to chromium exposure is well documented, several reviews of the literature (Cohen et al., 1993; Costa, 1997; ATSDR, 2000, Costa and Klein, 2006; Sedman et al. 2006) have summarized increases in the occurrence of other types of cancer attributed to chromium exposure.” To our knowledge, no NTP bioassay report has ever attempted to interpret and extend (in a speculative manner) the implications of epidemiological studies by citing selected reviewers’ opinions as to attributable cancer types. Indeed, several other literature reviews by authoritative individuals or groups have come to opposite opinions to those cited by Costa, Sedman and colleagues, and it seems inappropriate for NTP to rely heavily on their views but fail to discuss the relevant work of many other researchers or Agency workgroups, e.g., Paustenbach et al. (2003), Proctor et al. (2002), USEPA (1998), DeFlora (2000), and IARC (1990)

The discussion includes citations to limited studies associated with the same group of reviewers (i.e., Costa and his colleagues, e.g., Zhitkovich, Sugimura, Davidson), whose findings and interpretations cannot be considered directly relevant to humans, and certainly do not represent the scientific consensus on many chromium toxicology issues. Indeed, the recent review Costa and Klein (2006) was sharply criticized for containing errors and flawed interpretations on risk assessment of hexavalent chromium (Post and Stern, 2006). These reviews generally lean towards an interpretation of the literature that incorrectly suggests significant human health hazards at current USEPA drinking water standards for hexavalent chromium. It has been generally accepted for decades that the current MCL for chromium in drinking water should be adequate to avoid a human cancer hazard and the NTP data do not appear to put that conclusion into question.

Oral Detoxification Mechanisms. Rational limitations as to the range of plausible circumstances for long-term human exposures to hexavalent chromium in drinking water should be part of any discussion of the public health implications of the NTP cancer bioassay. For example, Kerger et al. (1996b) noted that humans are not likely to ingest to high dichromate concentrations in drinking water due to poor palatability and bright yellow coloration of contaminated water above about 1-4 mg Cr(VI)/L.

We recommend that the NTP report discuss more thoroughly the available literature that documents the role of hexavalent chromium reduction in gastric juice, blood, mucous, and tissues since these findings are directly relevant to interpretation of the isolated and species-specific tumor elevations noted in the dichromate bioassay. We also encourage NTP to include in the report a more thorough review of the scientific evidence on anatomical, physiological, biochemical, and toxicokinetic processes relevant to

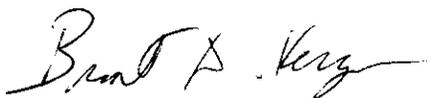
interpreting the species- and organ-specific tumorigenicity responses observed in the 2-year study.

As discussed above, hexavalent chromium has long been characterized as a 'portal of entry' tumorigen, with the oral pathway being an obvious exception to that premise (Proctor et al., 2002; De Flora, 2000). The biological and biochemical rationale (i.e., unique toxicokinetics of Cr(VI) compounds) that explains the absence of an oral tumorigenic response in the various animal species has been presented in many different papers (De Flora, 1978, 1982, 1984, 1985; De Flora and Boido, 1980; De Flora et al., 1987, 1988, 1989, 1997, 2006; De Flora and Wetterhahn, 1989; D'Agostini et al., 2002; Petrilli et al., 1985; Petrilli and De Flora, 1987, 1988; Kerger et al., 1996a, 1996b, 1997, 2002; Kuykendall et al., 1996; Finley et al., 1997; Mirsalis et al., 1996; Paustenbach et al., 1996, 2003; Corbett et al., 1998). These findings are consistent with the responses in key organs of mice and rats for the 2-year NTP study.

If the NTP report is to discuss the plausible applicability of these results to human health risk assessment, then we suggest that the report needs to discuss in greater detail the extensive peer-reviewed research showing the substantial reductive capacity of body fluids and tissues of the digestive tract and relevant dose limitations and toxicokinetics for oral exposures in humans (reviewed in De Flora, 2000 and Paustenbach et al., 2003). These inherent defense mechanisms are demonstrated to be effective at hexavalent chromium concentrations as high as 1 to 10 mg/L in humans and in animals. However, these same defense mechanisms undoubtedly operate most efficiently at plausible long-term drinking water exposures represented by hexavalent chromium concentrations below the USEPA MCL of 0.1 mg/L.

We appreciate your consideration of the above preliminary comments, and look forward to seeing the revised draft report.

Respectfully,



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Financial Interest Statement: Both signators are consulting scientists who have conducted research on chromium for the past 15 years. Much of that research was funded by private clients. Both have also been involved in conducting risk assessments of chromium, presented lectures at universities regarding chromium, and have provided expert witness testimony on chromium and several other chemicals. The signators were not funded by any client to submit these comments.

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