Cobalt-Tungsten Carbide Powders and Hard Metals Expert Panel Report

Part A – Peer Review of the Draft Background Document on Cobalt-Tungsten Carbide Powders and Hard Metals

The Report on Carcinogens (RoC) expert panel for cobalt-tungsten carbide powders and hard metals exposures met at the Sheraton Chapel Hill Hotel, Chapel Hill, North Carolina on December 9-10, 2008, to peer review the draft background document on cobalt-tungsten carbide powders and hard metals exposures and make a recommendation for their listing status in the 12th Edition of the RoC.

Members of the expert panel are as follows:

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One of the charges to this panel was to determine whether the information in the draft background document on cobalt-tungsten carbide powders and hard metals exposures is presented in a clear and objective manner, to identify any missing information from the body of knowledge presented in the document, and to determine the utility of the body of knowledge in the background document for drawing conclusions about the carcinogenicity of a candidate substance and for applying the RoC criteria for listing.

Following the discussion of all sections of the draft background document the expert panel reached a consensus concerning the critique of the draft background document, including its adequacy and any proposed revisions and voted 6 yes/0 no to accept the draft background document (with the proposed changes suggested by the expert panel). Therefore, the expert panel agreed that the background document is adequate for drawing conclusions about the carcinogenicity of cobalt-tungsten carbide powders and hard metals exposures and for applying the RoC listing criteria.

Following are the expert panel's proposed revisions for each section of the cobalt-tungsten carbide powders and hard metals exposures background document:

Document Title

- Suggest change to "Powders and Hard Metals of Cobalt-Tungsten Carbide"

Section 1: Introduction

- No comments

Section 2: Human Exposure

1. *Section 2.3 Biological indicators of exposure*
   - Pages 8-10 – place Section 2.3 after Section 2.4. Some of this information is described again in section 5.1, and it should be organized together.
   - Page 9, line 30 – add a new paragraph that discusses the data interpretation from the American Conference of Governmental Industrial Hygienists (ACGIH) Biological Exposure Index (BEI) documentation. A significant point made in this document is that the "high inhomogeneity in the data is attributed to differences in cobalt compounds used in the workplaces, to differences in the timing of urine collections, and to differences in analytical methods used in the studies." Include in this new paragraph data from five studies in the documentation of ACGIH's BEI for cobalt indicating that a urinary cobalt concentration between 14 and 26 µg/L or 18 µg/g creatinine is likely the result of occupational dust exposure to the Threshold Limit Value/Time-Weighted Average (TLV-TWA) of 20 µg/m^3. Cite Alexandersson and Lidums 1979; Alexandersson 1988; Ichikawa *et al.* 1985; Scansetti *et al.* 1985; Angerer and Heinrich 1988. Also include in this new paragraph that a cobalt concentration in blood between 1.1 and 2.3 µg/L is most likely due to occupational exposure to the TLV-TWA of 20 µg/m^3 (Alexandersson and Lidums 1979; Ichikawa *et al.* 1985; Lison, *et al.* 1994; and Angerer *et al.* 1985).
   - Page 10, line 12 – Add that the highest urinary concentrations were found in workers in the grinding workshop (median = 70.9 µg/g creatinine), production of tungsten carbide (median = 48.9 µg/g creatinine), and heavy alloy production (median = 21.6 µg/g creatinine).
2. *Section 2.4.2 Characteristics of hard-metal dusts across the production process*
   - Page 14, line 27 – add to end of paragraph "Lichtenstein *et al.* (1975) and Stebbins *et al.* (1992) sampled grinding operations for total and respirable airborne concentrations of cobalt and reported levels that exceeded the ACGIH TLV level."
3. *Section 2.4.4 Occupational exposure for various manufacturing processes*
   - Page 16, line 20 – insert the word airborne "…relatively low <u>airborne</u> exposure levels for cobalt…"
   - Page 16, lines 20-21 – After the sentence ending, "…moderately high levels for tungsten." add: "These high airborne tungsten carbide levels, however, were not associated with urinary levels. The highest tungsten carbide urinary levels were found among wet grinders (mean = 70.9 µg/g creatinine), and a mean airborne exposure level of 3.3 µg/m^3."
   - Page 16, line 21 – Add a new paragraph beginning with last sentence "Kumagai *et al.* (1996) similarly found…" Then add "Kumagai *et al.* reported that the within worker and between worker components ranged from 1.88 to 2.77 and 1.00 to 2.31, respectively. Because Kumagai *et al.* found that the geometric standard deviations (GSD) were low, this shows low day-to-day and between worker

variability. [Personal exposures to workplace agents are known to be highly variable and conform to a lognormal distribution.] Kumagai *et al.* reviewed the components of variance across a variety of job groups within a hard metal tool manufacturing facility by collecting 935 samples from 275 workers. [If large variance exists, then it is less possible to detect significant associations between exposure and disease.] But Kumagai *et al.* found that the geometric standard deviation (GSD) was less than 3.0, showing low variability."

4. *Tables 2-1 and 2-2, page 17*
   - Add "N" in footnotes for both tables.

5. *Table 2-3, pages 21-23*
   - Add notes that identify the data as pre-sintering/sintering (Step 2) or post-sintering (Step 3) in title.
   - Add data from Table 1 (page 5) of cobalt and inorganic compounds (ACGIH).

6. *Section 2.4.6 Exposure levels during finishing of sintered hard-metal products*
   - Page 25, line 24 – Add, "Stebbins *et al.* (1992) reported that of 3/9 air samples, tungsten airborne concentrations ranged from 410-710 $\mu g/m^3$ among the dry and wet grinding operations."
   - Page 26, line 17 – change to "…engineering controls, in particular local exhaust ventilation systems, and their long-term effectiveness in controlling airborne cobalt levels."
   - Page 27, lines 25-27 – revise the sentence to "This study supports the assertion that end use is expected to result in less exposure than either manufacturing or maintaining the hard metal products. The exposure assessment included three control machine shops that did not grind hard metals. The mean cobalt level for hard metal exposed workers (N = 141 samples from 78 exposed workers) was 20.2 $\mu g/m^3$ (range: 0.7-279 $\mu g/m^3$) and 7% were above 50 $\mu g/m^3$. The machining (control) shops had low cobalt concentrations (mean = 1.2 $\mu g/m^3$; range = 0.4-4 $\mu g/m^3$)." Recommend deleting "supporting the assertion that end-use is expected to result in less exposure than either manufacturing or maintaining the hard-metal products." as it pertains to saw mills.

7. *Table 2-4, pages 28-32*
   - Page 28 – In title, add notes that identify the data as pre-sintering/sintering (Step 2) or post-sintering (Step 3).
   - Page 32 – Add new footnote to refer reader to stellite definition in glossary and state that stellite data are not included.

8. *Section 2.5 Environmental levels and general population exposure*
   - Pages 35-37 – Review literature for potential information on "take home" exposure to children from parental occupational exposures.

9. *Section 2.6.2 Guidelines*
   - Page 38, line 11 – For the ACGIH BEI's, refer the reader to the three ACGIH TLV documents: cobalt and inorganic compounds; tungsten and compounds; cobalt, all inorganic forms, except cobalt oxides (ACGIH 2001a,b,c).

10. *Section 2.7 Summary*
    - Page 38-39 – Add information on exposure during grinding and sharpening by "end users" at saw mills, etc.

Section 3: Human Cancer Studies

1. *Introduction*
   - Page 41, line 4-7 – "IARC concluded that there was limited evidence of carcinogenicity of cobalt in hard metals in humans, based on epidemiological studies showing an increased risk of lung cancer among workers exposed to hard-metal dust containing cobalt and tungsten carbide….": Insert "with evidence of an elevation of risk with increasing exposure" after "tungsten carbide" (See p.130 of IARC 2006.)
   - Page 41, line 19 – Move second sentence to beginning of paragraph: "No adequate epidemiological studies have been conducted that evaluated the relationship..." then keep the sentence "As mentioned in Section 2…" then delete "Elevated levels…" Add "A cross-sectional case comparison study was conducted (Rubin *et al.* 2007); but found no relationship between leukemia occurrence and urinary tungsten levels. However, the study did not assess exposure to tungsten carbide, cobalt, or hard metals."
   - Page 42, line 10 – "The primary tissue site relevant to potential carcinogenicity of exposure to cobalt-tungsten carbide powders and hard metals": Replace "tissue" with "organ."
   - Page 42, lines 10-12 – The concluding clause of the sentence stating "The primary tissue site relevant to potential carcinogenicity of exposure to cobalt-tungsten carbide powders and hard metals is the lung, through inhalation exposure to hard-metal dust" could be misleading. Delete "…exposure to hard metal dust."
   - Page 42, lines 12-16 – Delete this sentence "The leading cause of lung cancer is smoking; however, a number of known or suspected carcinogens (such as polycyclic aromatic hydrocarbons (PAHs), nitroamines [nitrosamines]….",

2. *Section 3.2 Cohort studies (general)*
   - For the four studies, indicate the gender of the studied population.
   - When reporting results of each epidemiologic study, it is useful to give the total number of deaths from all causes, and, when available, total person years.

3. *Section 3.2.1 Hogstedt and Alexandersson (1990)*
   - Page 43, lines 6-7 – "Exposure was assessed retrospectively using measurements from the 1950s and from the 1970s, and expert knowledge of working conditions.": Insert "and how they evolved over the relevant decades" after "conditions."
   - Page 44, lines 7-9 – "The lung-cancer SMRs increased again when the analysis was further restricted to workers with duration of exposure greater than 10 years,": Insert: "and > 20 years latency," after "years."
   - Page 44, lines 11-14 – "However, only when the analysis combined all exposed workers with time since first exposure greater than 20 years and duration of exposure greater than 10 years did the excess lung-cancer mortality become statistically significant (SMR = 2.78, 95% CI = 1.11 to 5.72, 7 deaths).": Delete "However, only." The lack of statistical significance in previous sentences is obvious and is burdened by small numbers. This phrase over-emphasizes the importance of statistical significance testing here.
   - Page 44, lines 16-18 – "In both the total cohort and Factory A, SMRs were somewhat higher among workers with exposure times from 1 to 4 years than workers with 5 or more years of exposure.": Insert "(where exposures were highest)" after "Factory A." Also, insert "though these differences were not statistically significant." after "exposure" at the end of the sentence.

- Page 44, line 18 – Add that in addition 4 of the 292 deaths from pulmonary fibrosis were observed in this cohort, which may represent pneumoconiosis associated with exposure to hard metal dust.
- Page 45, lines 10-11 – “Given these small numbers, the power to detect an excess was very small.” Insert “statistically significant” between “detect a[n]” and “excess.”
- Page 45, line 11 – add after “excess” “Despite the limited statistical power associated with the sub-group with > 20 years latency and > 10 years employment duration, the elevated lung cancer SMR associated with this sub-group was statistically significant.”
- Page 45, line 18 – add: “The choice of referent death rates is a general problem with SMRs, which can be solved by conducting analyses within the cohort.”

4. *Section 3.2.2 Lasfargues et al. (1994)*
   - Page 47, line 23 – In sentence “Nonetheless, a significantly elevated SMR was observed in the high-exposure group.” Insert: "for lung cancer" after "SMR."
5. *Section 3.2.3 Moulin et al. (1998)*
   - Page 48, line 12 – Mention that there was a 15% loss to follow-up. The authors stated that most of the workers that were lost were foreign born.
   - Page 49, lines 11-13 – “However, there were exposure measurements available from 1971 to 1974 for certain workplaces of certain factories.”: Change 1974 to 1994. On page 243 of the paper, it says that cobalt measurements were taken between 1971-1983 and 1982-1994.
   - Page 50, lines 5-6 – “No excess mortality was found for non-malignant respiratory diseases.”: Add “though 3 workers died from pneumoconiosis. The SMR for emphysema and chronic bronchitis was 0.21.” after “respiratory diseases.”
   - Page 50, lines 18-22 – Change text “Possibly even more important is potential confounding by exposure to other agents…” to “There is also potential for confounding from other agents.”
6. *Wild et al. (2000)*
   - Page 51, lines 7-8 – “Exposure to smoking was obtained from occupation health department records.”: Insert, "though limited information was available about smoking prior to 1978." after "records."
   - Page 52, lines 1-8 – Add the SMR for ‘only employed’ post sintering hard metal production: SMR = 1.13, 95% CI = 0.31 to 2.89.
7. *Section 3.3 Discussion*
   - Page 53, lines 12-15 – Delete bracketed comments on the overlaps among the three French studies. Add the bracketed statement, “[The Lasfargues *et al.* (1994) cohort is small and is completely included in the study by Moulin *et al.* (1998), which has 2 more years of follow-up and improved exposure assessment. Although the cohort studied by Wild *et al.* (2000) is also included in the report by Moulin *et al.*, the Wild *et al*. paper adds information through more detailed exposure-response analyses and improved control for confounding.]”
8. *Section 3.3.1 Confounding*
   - Page 54, line 3 – At end of paragraph, add “The lack of excess non-malignant respiratory disease deaths, especially chronic obstructive lung disease or emphysema, and other smoking related cancers, suggests limited potential for confounding by smoking in these studies.”
   - Then add “Both the Moulin *et al.* and Wild *et al.* studies used internal analyses (via nested case-control analysis in the former and Poisson regression in the latter), which are usually less susceptible to confounding relevant to comparisons to external referent populations.”

9. *Section 3.3.2 Exposure-response relationships*
    - Page 54, line 4 – Change title to "Exposure Assessment and Exposure Response Relationships"
    - Add the following discussion on measurement error: "As in most occupational studies, a major limitation of these studies is the potential for error in measuring exposure to cobalt-tungsten carbide powders and hard-metal powders. None of the studies have quantitative exposure estimates for individual workers. The exposure assessments in the Swedish study (Hogstedt and Alexandersson 1990) and the small French study (Lasfargues *et al.* 1994) are relatively crude, and workers are classified into either two or four exposure categories. More comprehensive exposure assessments were performed in the studies by Moulin *et al.* (1998) and Wild *et al.* (2000). These studies used the same job exposure matrix (JEM), based on plant visits, historical records, and interviews, and calculated semi-quantitative estimates of exposure to hard-metal dust (both sintered and unsintered), cobalt alone, and tungsten carbide alone. The semi-quantitative exposure scores from the JEM were significantly correlated with the available measurements of cobalt in air, but exposures were not uniform within groups (see Section 3.2.3). The sample sizes within exposure groups are also small, which reduces the precision of the estimated exposure level, particularly in groups 6 and 7. In most cases, such random errors in estimating exposure lead to attenuation of observed exposure-response relationships toward the null value. Exposure misclassification also decreases the power to detect a positive exposure-response relationship. Another major limitation is that the exposure assessment was semi-quantitative rather than quantitative and thus, it is not possible to estimate the risk per unit of exposure. Semi-quantitative exposure assessments also limit the ability to separate the effects of cobalt and tungsten carbide."
    - Page 54, lines 11-13 – "Moulin *et al.* reported that the odds ratio (OR) in the nested case-control study increased significantly with increasing cumulative exposure (OR = 4.13, 95% CI = 1.49 to 11.5, with 23 cases in the highest exposure quartile).": insert "unweighted" after "increased significantly with increasing..."
    - Page 54, line 24 – Add at end of section: "A strength of the Moulin *et al.* and Wild *et al.* studies is that they focused on internal comparisons (via nested case-control analysis in the former and Poisson regression in the latter). As mentioned above, internal analyses are usually more informative in assessing causality than comparisons to external referent populations."

10. *Section 3.3.3 Human cancer studies evaluating other exposures to cobalt*
    - Page 54, line 25 – Change title to add "and tungsten."
    - Add a statement that acknowledges that there is not an adequate database to evaluation the potential carcinogenicity of tungsten in humans.

11. *Section 3.4 Summary*
    - Page 55, lines 26-27 – "Both Wild *et al.* and Moulin *et al.* attempted to control for smoking and/or other occupational exposure.": insert "and Lasfargues *et al.* (1994) study provided data that showed that the study population differed little in smoking habits from a national sample of French men." after "other occupational exposure".

Section 5. Other Relevant Data

1. *Section 5.1.2 Experimental animals*
    - Page 67, line 13 – Indicate that the time point at which urine was sampled in the study of Lasfargues *et al.* (1992) was 24 hr and that the cobalt and cobalt-tungsten carbide were administered in equivalent cobalt amounts per kg bw.

- Page 67, line 17 – In the study of Lison and Lauwerys (1994), indicate that cobalt and cobalt-tungsten carbide were administered in equivalent cobalt amounts per kg bw and that urine samples were collected after 24 and 48 hr.

2. *Section 5.1.3 In vitro studies*
   - Page 67-68 – Add information on solubility from Stopford *et al.* (2003) and Lombaert *et al.* (2004).

3. *Section 5.2.1 Humans – Contact dermatitis*
   - Page 70 – Add information "The allergic dermatitis observed after sensitization due to contact with cobalt or its compounds is a type IV or delayed type hypersensitivity reaction (Nordberg 1994)."
   - Page 70, lines 10-16 – Respiratory effects: Add reference Day *et al.* (2008).

4. *Section 5.2.2 Animal models of hard-metal toxicity:*
   - Consider adding the following studies:
     1. Schepers (1955a and b): instillation of cobalt metal alone versus mixed with tungsten carbide, respectively, in guinea-pigs.
     2. Kaplun & Mezencewa (1960): presence of tungsten carbide increased the lung toxicity in rats observed for cobalt due to higher solubility.
     3. Tozawa *et al.* (1981): pre-sintered cemented carbides administered to rats caused fibrotic foci in lungs.
     4. Rengasamy *et al.* (1999): effect of hard metals on nitric oxide pathways

5. *Section 5.2.3 Cytotoxicity studies*
   - Add a description of Lison and Lauwerys (1995): Comparison of cytotoxicity of cobalt-tungsten carbide with other combinations of cobalt and metallic carbides.
   - Page 76 – Roesems *et al.* (2000): add that "leachate" was produced by placing the particles in an insert above the cells.

6. *Section 5.3.2 Mammalian systems* in vitro
   - Page 83, lines 13-14 – Change sentence "The extent of DNA damage is determined" to "The extent of DNA damage is generally determined by % comet tail intensity, tail length or by tail moment (i.e., the product of comet tail length and tail intensity)."
   - Page 83, line 28 – Note that the lesions detected by the Fpg enzyme represent only a fraction of the possible DNA lesions induced by oxidative stress (i.e., mainly oxidized purines like 8-OHdG).
   - Page 84, lines 10-12 – in De Boeck *et al.* (1998) cobalt chloride ($CoCl_2$) is demonstrated to induce DNA strand breaks in the alkaline comet assay. Delete the sentence and reference to De Boeck *et al.* (1998).
   - Page 84, lines 12-16; page 88, lines 23–25; page 102, lines 28–29; and also page ix, lines 23-24 – "Cobalt ions contribute to aneugenic effects (as evident from micronucleus formation)." Change sentence on page ix, lines 23-24 and page 102, lines 28-29, "Cobalt ions have genotoxic effects as evidenced from DNA strand breaks in the comet assay, inhibition of DNA repair, and micronucleus formation."
   - Page 84, lines 12-16 – Change sentence to "The mechanisms for the genotoxicity of cobalt-tungsten carbide particle may include clastogenic effects mediated by ROS produced at the surface of these particles and/or aneugenic effects mediated by Co(II) ions released from these particles, and possibly others."

- Page 88, lines 23-25 – Change sentence to “The genotoxic effects of cobalt-tungsten carbide particles may be due to ROS produced at the solid-liquid interface and/or from ionic cobalt forms dissolved in biological media.”

7. *Section 5.3.3 Rat* in vivo *systems*
   - Page 85, line 9 – Add to the study of De Boeck *et al.* (2003b): “the authors related this to the lack of systemic exposure to the particles (bleomycin gave increased levels of micronuclei in PBMC although not statistically significant).”
   - Page 85, lines 9-10 – “BAL cells of treated animals showed statistically significantly lower levels of DNA migration than controls”. Add the following: “The authors stated that changes in the relative proportion of exposed versus non-exposed cells in the BAL fluid could have contributed to this observation.”
8. *Section 5.4 Mechanistic studies and considerations*
   - Add a discussion about the general inhibition by cobalt ions of the dioxygenase enzymes on page 88. The new text should discuss the following information. There is limited information about the mechanism of how cobalt-tungsten carbide may cause toxicity and cancer. Although it is not well presented in the literature a major target of cobalt ions is the iron containing dioxygenase enzymes. Some discussion of this is found with reference to the fact that cobalt ions can stabilize HIF-1 alpha transcription factor. However the reason for this is thought to be due to cobalt ion inhibition of the prolyl hydroxylase that is responsible for signaling degradation of HIF-1 alpha. This prolyl hydroxylase is the target for cobalt ions because cobalt may displace the bound iron at the active site of this enzyme and the enzyme is inactive with cobalt ions there in place of iron. This effect is also found for other dioxygenase enzymes which include the newly discovered histone demethylases, the Alb B type DNA repair enzymes (the human homologue is ABH2) to name but a few. There are data to show inhibition of prolyl hydroxylase by cobalt ions: (Kim *et al.* 2006, Hirsilä *et al.* 2005). There are numerous papers on the subject of metal ion inhibition of dioxygenase enzymes and a discussion is needed about cobalt ion inhibition of this class of enzymes, and that the resultant effect of the inhibition is to stabilize HIF-1 alpha, increase histone methylation which can activate (H3K4) or silence genes (H3K9) and inhibit DNA repair (ABH2).) This is a likely mechanism by which cobalt ions exert their carcinogenic and toxic effect in cells. Since these enzymes use ascorbic acid, oxidative stress and depletion of ascorbate is another possible route to inhibit these enzymes (Salnikow *et al.* 2004). This paper demonstrates the ability of cobalt to quickly deplete ascorbate, a major cellular antioxidant. This may increase oxidative damage and contribute to dysregulation of the expression of HIF-inducible genes. Ascorbate depletion may also impair certain mechanisms of DNA repair (base dealkylation) and nickel ions which act like cobalt ions have been shown to inhibit the histone demethylases (Chen *et al*. 2006).
   - Particulate metals such as insoluble cobalt compounds and tungsten carbide can be phagocytized by non-macrophage cells in the respiratory tract. This will lead to intracellular metal accumulation at very high levels and even a small amount of dissolution of the metal ions may generate high soluble metal ion levels in cancer target cells. This issue needs to be addressed (Costa *et al.* 1982, Lombaert *et al.* 2004).
   - Add discussion of solubility of presintered and sintered cobalt-tungsten carbide in biological fluids (they are similar taking particle size differences into consideration) (Stopford *et al.* 2003).
   - Page 88, lines 26-30 – Give more details of study of Lombaert *et al.* (2004): effect of cobalt-tungsten carbide on apoptosis induction was not additive at short treatment time using an early apoptosis marker; effect was additive at 24 hours

and a later apoptosis marker. Lombaert *et al.* (2004) also used PBMC. Add bracketed comment that peripheral blood lymphocytes *in vitro* are highly susceptible to chemical-induced apoptosis.

9. *Section 5.5.2 Genotoxicity of cobalt compounds*
   - Page 97, line 11 – Among the possible mechanisms of action, interference of cobalt ions with DNA repair processes is only briefly highlighted (Hartwig *et al.* 2002). Since this constitutes an important mechanism, relevant also for cobalt-tungsten carbide, it would be justified to elaborate more in detail on this subject as follows: "Hartwig *et al.* (2000, 2002) reported that a number of carcinogenic metals, including cobalt, interfere with DNA repair processes at low, noncytotoxic concentrations. Repair of DNA damage is vital for maintaining genomic integrity; thus, inactivation of DNA repair may be an important mechanism of metal-related carcinogenicity. Cobalt compounds are comutagenic in bacteria and mammalian cells, affect both the incision and the polymerization of repair patches, and disturb cell-cycle progression and control in response to ultraviolet C radiation. Interactions with zinc finger proteins, which are involved in DNA binding and protein-protein interactions, were identified as potential molecular targets for metal ions. Cobalt inhibited mammalian xeroderma pigmentosum group A protein (XPA) and the poly(adenosine diphosphate-ribose)polymerase (PARP), but not the bacterial formamidopyrimidine-DNA glycosylase (Fpg). XPA is essential for DNA damage recognition during nucleotide excision repair and PARP directs repair enzymes to the sites of damage and plays a role in apoptosis. In addition, the p53 protein is another zinc-dependent transcription factor involved in cell-cycle control and apoptosis. Cobalt also inhibits its DNA-binding activity."
   - Regarding the promutagenic oxidative DNA damage by cobalt compounds it should be stressed in the background document that this kind of damage has been found not only *in vitro,* but also in organs of cobalt-exposed animals (compare Nackerdien *et al.* 1991 with Kasprzak *et al.* 1994).
   - Page 97, line 11 – Add the following as a new paragraph: 'Hartwig *et al.* (2000) reported that cobalt compounds damage DNA in the presence of reactive oxygen species. Cobalt ions and other carcinogenic metals are known to perform redox reactions in biological systems (Beyersmann and Hartwig 2008). These reactions result in reactive oxygen and nitrogen species *in vivo* and *in vitro* in mammalian cells. Fenton and Haber-Weiss-type reactions are likely responsible for the formation of hydroxyl radicals that can cause oxidative damage to lipids, proteins, and DNA. Therefore, oxidative stress is not the sole cause for metal carcinogenesis but is likely a contributing factor. Nackerdien *et al.* (1991) investigated the ability of Co(II) ions in the presence of hydrogen peroxide to cause chemical changes in DNA bases in chromatin extracted from human K562 cells. The typical hydroxyl radical-induced products of DNA bases were identified. Hydroxyl radical scavengers resulted in partial inhibition of product formation, while chelation of Co(II) ions with EDTA resulted in an almost complete inhibition of product formation. In an *in vivo* study, Kasprzak *et al.* (1994) reported oxidative DNA base damage in renal, hepatic, and pulmonary chromatin of rats after intraperitoneal injection of 50 or 100 µmol Co(II) acetate. The bases were typical products of hydroxyl radical attack on DNA and showed increases of 30% to more than 200% over control levels.'
   - Page 97, line 16 – Add a reference for the statements in lines 12-16.

10. Table 5-4, pages 97-100

- General: better define the following endpoints: DNA strand breaks, DNA migration, DNA damage, DNA breakage in terms of the test system used. Add the assay to the end point column.
- Replace De Boeck *et al.* (2003c), which is a review article, with the original references. (These include p. 99, rows 1, 3, 17, 18; p. 100, rows 1–3.)
- Page 99, row 3 – Add footnote to De Boeck *et al.* (2003c) (that aneuploidy was determined by karyotyping.
- Add info from Colognato *et al.* 2008 as follows:

Cobalt compound	Test system	End point	Result	Reference
Cobalt nanoparticles	Human peripheral lymphocytes	DNA damage Binucleated micronucleated cells	+ +	Colognato *et al.* 2008
Cobalt chloride	Human peripheral lymphocytes	DNA damage Binucleated micronucleated cells	– +	

11. Section 5.5.3 Toxicity of cobalt compounds or tungsten carbide

- Section 5 of this document provides nearly complete relevant information on the pathogenic effects of cobalt and its compounds alone, but only very limited information on tungsten and its compounds (including tungsten carbide). This, generally, reflects the scarcity of applicable data in publicly available publications, especially those on tungsten carbide, creating the impression that in the cobalt-tungsten carbide powders and hard metals, the cobalt moiety is the major, if not the only, villain. To better balance the information, we should include the data from: Wennig and Kirsch 1988, Wei *et al.* 1985, Miller *et al.* 2002, Miller *et al.* 2004. Proposed text describing these studies is provided below and in item 12 (Section 5.5.4).
- Change Section 5.5.3 title to "Toxicity of tungsten carbide and tungsten compounds" and move the paragraph on cobalt to the previous section.
- Add the following text as a new paragraph at the end of Section 5.5.3: "The experimental toxicity of tungsten compounds was reviewed by Wennig and Kirsch (1988). Soluble compounds are rapidly absorbed following ingestion and excreted in the urine (40%) and feces (58%) within 24 hours in rats. The soluble tungsten salt (Na_2WO_4) was reported to be moderately toxic by ingestion. Acute effects of this compound included central nervous system disturbances, diarrhea, respiratory failure, and death. Chronic exposure in rats resulted in reduced body weight, reduction of albumin, SH groups, γ-globulin, and uric acid in blood, and affected sperm motility. Tumor induction, body weight, and survival were not affected in rats administered 5 mg/L Na_2WO_4 in drinking water for life. Hard-metal workers had no allergic reactions to Na_2WO_4 in patch tests. Another compound, tungsten hexafluoride (WF_6) reacts with water to release hydrogen fluoride and can cause respiratory tract irritation, laryngitis, bronchitis, cyanosis, and pulmonary edema. *In utero* exposure to tungsten compounds resulted in embryo lethality and disturbance of skeletal ossification in rats and increased frequency of resorptions in mice."

- Include a discussion of solubilization of tungsten from cobalt/tungsten carbide from Lombaert *et al.* (2004). In this paper, solubilization of cobalt and tungsten is determined in culture medium (containing 15% serum) by ICP-MS. The purpose was to relate solubilization to the actual *in vitro* cell conditions used in the genotoxicity/apoptosis experiments. As expected, tungsten was only marginally solubilized (2%) in 24 hr compared to cobalt, which was 74% solubilized at 24 hr. The tungsten carbide particles were internalized by monocytes.

12. Section 5.5.4 Carcinogenicity and other studies of a tungsten-cobalt alloy

- Page 100, line 16 – Change subheading 5.5.4 to the "Carcinogenicity of tungsten compounds."
- Page 101, line 6 – Add the following text as a new second paragraph: "Wei *et al.* (1985) investigated the effects of molybdenum and tungsten on mammary carcinogenesis in SD rats. Female rats (35 days old) were randomly divided into four groups. Groups 1 through 3 were fed a nutritionally adequate diet and demineralized water, while group 4 was given the same diet with 150 ppm tungsten added to the drinking water. At 50 days of age, rats in groups 2 through 4 (22 to 24 animals) were injected via the tail vein with 5 mg/100g body weight of *N*-nitroso-*N*-methylurea (NMU). Group 1 (10 animals) received injections of the saline vehicle and served as the untreated controls. One week after treatment with NMU, 10-ppm molybdenum was added to the drinking water of group 3. Animals were sacrificed 125 days or 198 days after NMU treatment. Body-weight gains were slightly lower in the NMU-treatment groups compared with the control. Mammary tumors appeared earlier in the tungsten treatment group (group 4). There was a significant increase in the incidence of mammary carcinomas in group 4 (79.2%) compared with group 2 (50%) after 125 days, but at 198 days, the incidences were similar (90.5% group 2 and 95.7% group 4). The tumor incidence in group 3 was 45.5% at 125 days and 50% at 198 days. No tumors occurred in the untreated controls. [The potential promoting effect of tungsten on nitrosamine-initiated cancer could be important because cutting fluids used to lubricate hard metal cutting tools may contain nitrosamines.]"
- Also, in this paper (Wei *et al.* 1985), the authors mention that breast cancer mortality among residents of tungsten mining areas in China are markedly higher than the national average (no known follow-up to this study).
- Page 101, lines 6-14: Add the following text to supplement the Miller *et al.* (2001) study. Miller *et al.* (2002, 2004) conducted further tests with the tungsten, nickel, and cobalt mixture (designed to simulate alloys used in military applications). They used a human osteosarcoma cell model. Cultured cells were incubated with the metal powder for 24 hours to assess morphological cell transformation. Cytogenetic analyses also were conducted to determine micronuclei, SCE, and DNA single-strand breaks (Miller *et al.* 2002). Miller *et al.* (2004) investigated the ability of the tungsten, nickel, and cobalt mixture and the pure metals to induce stress genes in 13 different recombinant cell lines from human liver carcinoma cells. These studies indicated that the tungsten mixture could transform human cells to the tumorigenic phenotype through induction of DNA and chromosome damage. The mixture showed dose-related induction of GSTYA, hMTIIA, p53RE, FOS, NFκBRE, HSP70, and CRE promoters. Each of the individual metals showed a similar pattern of gene induction, but at a significantly lower level than the mixture.

Appendix A

Revise as follows:

- Page 137, line 23 – Delete from “A military aviation...” to Page 138, line 10.
- Page 139, line 13 – Delete starting “Urinary levels of arsenic…” to Page 140, line 10 and briefly summarize the findings of “other factors” section.

Suggested Additional Literature:

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Miller AC, Brooks K, Smith J, Page N. 2004. Effect of the militarily-relevant heavy metals, depleted uranium and heavy metal tungsten-alloy on gene expression in human liver carcinoma cells (HepG2). *Mol Cell Biochem* 255(1-2): 247-56.

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Redacted

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Max Costa, Ph.D., Chair — Date