Responses to Public Comment on the Draft Cancer Inhalation Unit Risk Factors for Cobalt and Cobalt Compounds

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

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On March 8, 2019, the Office of Environmental Health Hazard Assessment (OEHHA) released the draft document, *Cobalt and Cobalt Compounds Cancer Inhalation Unit Risk Factors* to solicit public comment. Responses to comments received on the draft Cobalt and Cobalt Compounds Cancer Inhalation Unit Risk Factors (IURs) are provided here.

Background

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Hot Spots) (Health and Safety Code Section 44360(b)(2)). OEHHA developed a Technical Support Document (TSD) in 2009 to respond to this statutory requirement that lists and describes cancer potency factors used in the Hot Spots program. The TSD presents methodology for deriving cancer potency factors. In particular, the methodology explicitly considers possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Chapter 731, Statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*). These guidelines have been used to derive cancer potency factors for cobalt metal and cobalt sulfate heptahydrate.

Comments on the Draft IURs for cobalt and cobalt compounds were received from:

- ToxStrategies, Inc.
- Cobalt Institute
- Color Pigments Manufacturers Association

Responses to Comments Received from ToxStrategies, Inc.

ToxStrategies Comment 1:

1. Water solubility is not the correct measure for categorizing cobalt compounds.

The categorization of cobalt and cobalt compounds by water solubility is inappropriate and is not supported by inhalation bioaccessibility data for cobalt compounds. We are concerned that, without further differentiation and clarification in the OEHHA document, these categories will lead to significant confusion and errors in risk assessment, such that cobalt in steel will be confused with pure cobalt metal. We recommend that cobalt forms be differentiated based on lung fluid bioaccessibility rather than water solubility.

Cobalt metal, in its pure form such as that administered in the NTP (2014) study, should not be categorized with the vast majority of water-insoluble cobalt compounds. Notably, both cobalt metal and cobalt sulfate are readily accessible in artificial lung fluids, and they represent highly bioavailable substances. Categorization based on water solubility is likely to result in misclassifying other water-insoluble forms of cobalt, particularly cobalt in alloys such as stainless steel, and cobalt in ceramics, as being carcinogenic in the lung and incorrectly assessing them in air toxics risk assessments.

Uses of cobalt in the United States are shown in **Table 2** (re-created from data presented in NTP 2016b). Cobalt is used in various industrial applications as a colorant, catalyst, and as a drying agent for glass, ceramics, paint, inks, feed supplements, batteries; it is used to produce alloys or composites (NTP 2016b). However, as evidenced in **Table 2**, the primary use of cobalt is in steel-related alloy applications. Hence, cobalt is used primarily in forms that are water insoluble, but not nearly as bioaccessible and bioavailable as cobalt in the pure metal form. We are concerned that errors will result in applying the IURs to forms of cobalt that, like cobalt in stainless steel, are water insoluble but do not behave biologically in the same manner as pure cobalt metal.

Table 2. Use patterns for cobalt in 2012 for United States (recreated from Table 2-3 of NTP 2016b)

End Use	Consumption (Metric Tons Cobalt Content)	% Total Consumption
Super Alloys	**4,040**	**48**
Chemical and ceramic	**2,300**	**27.3**
Cemented carbides	**774**	**9.2**
Other alloys*	**699**	**8.3**
Steels	**548**	**6.5**
Miscellaneous and unspecified	**63**	**0.7**

***** Includes magnetic, nonferrous, and wear-resistant alloys and welding materials

Cobalt in alloys is not bioavailable like cobalt metal or water-soluble cobalt compounds such as cobalt sulfate (Hillwalker and Anderson 2014). It should be noted that NTP's 14th RoC lists cobalt sulfate and cobalt-tungsten carbide powders and hard metals as reasonably anticipated to be human carcinogens, and the RoC Monograph on cobalt and cobalt compounds reached the same conclusion based on animal and mechanistic data (NTP 2014, 2016a). Notably, cobalt-containing alloys were not classified with these compounds. On Page 2, OEHHA states, "The cobalt IURs do not apply to cobalt alloys (e.g., cobalt-tungsten hard metal dust) or the cobalt-containing essential nutrient vitamin B12." We agree with this statement, but we request additional clarification that cobalt in steel and super alloys be specifically excluded or that the categorization of cobalt and cobalt compounds be based on lung bioaccessibility. This is an important clarification because cobalt-tungsten hard metals are not representative of the forms of cobalt that occur in stainless steel and super alloys.

Response to ToxStrategies Comment 1:

The commenter is asking for changes in the categorization of cobalt and cobalt compounds in the Cobalt and Cobalt Compounds Technical Support Document (TSD) such that, 1) cobalt forms be differentiated based on lung fluid bioaccessibility rather than water solubility, and 2) that cobalt alloys in addition to cobalt-tungsten hard metals

Regarding part 1 of the comment, OEHHA believes that categorizing cobalt compounds using water solubility and lung fluid bioaccessibility are both important factors for deciding which IUR value applies to a particular cobalt compound. The toxicological database indicates that the important physiological factor for carcinogenicity of insoluble forms of cobalt is whether the inhaled cobalt compound will be taken up by lung cells in particle form by endocytosis, and then solubilized in lysosomes. Inhaled cobalt metal particles are mainly distributed to the cells in this manner, due to insolubility in water, and then dissolve in the acidic environment of lysosomes. The National Toxicology Program (NTP) carcinogenicity studies suggest that this type of cellular distribution of cobalt metal results in a nearly 10-fold increase in cancer potency relative to the inhalation and cellular uptake of soluble cobalt compounds. By extension, OEHHA proposes to use the IUR for cobalt metal for other insoluble cobalt compounds based on the similar cellular uptake pathway.

OEHHA is using water solubility as a "first cut" in assessing the carcinogenicity potential of a cobalt compound. As stated in the OEHHA cobalt TSD, "Water-soluble cobalt compounds reaching the alveoli following inhalation will dissolve in the alveolar lining fluid and release the cobalt ion (Kreyling et al., 1986; Stopford et al., 2003). Water-insoluble cobalt compounds (e.g., cobalt oxides) and cobalt metal reaching distal airways and alveoli may dissolve intracellularly in the acidic environment of lysosomes (pH 4.5 to 5) following uptake via endocytosis by macrophages and other epithelial cells (Kreyling et al., 1990; Ortega et al., 2014). These findings are supported by extensive *in vitro* and *in vivo* evidence.

The water solubility of a compound or metal is one of the most common measures used to describe its physical properties. As such, water-solubility information for various cobalt compounds is more common than alveolar and interstitial lung fluid solubility data, so it would be negligent for OEHHA to ignore the water solubility data. NTP (2016) takes a similar approach by presenting the water solubility of cobalt metal and cobalt compounds, alongside the bioaccessibility data in lysosomal fluid (on page 2 of the Report on Carcinogens).

Regarding Part 2 of the comment, OEHHA had already explicitly stated in the document that the cobalt IURs do not apply to cobalt alloys. However, the document has been revised to more definitively exclude other cobalt alloys, in addition to cobalt-tungsten hard metals, from the IURs derived and designed for cobalt compounds. Cobalt-tungsten hard metals, as summarized in the cobalt TSD, exhibit unique properties that suggest the interaction between the two metals produces activated oxygen species that is markedly greater than that produced by cobalt metal alone (Lison et al., 1996). Zanetti and Fubini (1997) describe that the two metals together act like a new

compound with different physico-chemical properties from those of cobalt and tungsten alone. Attempting to use the cobalt metal IUR to assess the carcinogenicity of cobalt-tungsten hard metal dust may underestimate the carcinogenicity of this alloy. Thus, it appears appropriate to categorize cobalt metal alloys separately from cobalt metal and compounds when assessing cancer and noncancer risk, as recommended in Hillwalker and Anderson (2014).

ToxStrategies Comment 2:

1.1 Cobalt metal should be recognized as bioaccessible and bioavailable in the lung.

Cobalt metal is soluble in dilute acids and biological fluids, including lung cytosol, plasma, and intracellular lysosomal fluids. NTP stated, “Cobalt metal particles have been found to be 100% bioaccessible (i.e., dissolving to release cobalt ions) in both artificial gastric and lysosomal fluids” (NTP 2016b). Dissolution in lysosomal fluids is designed to represent intracellular solubility in the lung. Dissolution in lysosomal fluid is assessed to evaluate the potential for release of ions in the nucleus and is applicable for metals that are insoluble in the neutral conditions of alveolar and interstitial fluids but may be transported into lung cells by means other than simple dilution.

It is critical to consider that bioaccessibility and bioavailability of metals depend on the micro-environment in which the metal compound resides. The insolubility of cobalt metal in water does not mean that it has limited bioaccessibility and bioavailability in biological fluids. As evidenced in Stopford et al. (2003), solubility of cobalt metal in lysosomal fluid is similar to that of cobalt sulfate heptahydrate [data not shown here; refer to the submitted comments]. This is contrary to the limited bioacessibility of cobalt in alloys reported in Hillwalker and Anderson (2014) and ToxStrategies (2017) [OEHHA note: data not shown here; refer to the submitted comments]; these data are discussed further in section 1.3. It is evident that both cobalt metal and cobalt sulfate heptahydrate represent highly bioavailable forms of cobalt unlike cobalt in alloys.

Moreover, water solubility is a poor surrogate for solubility of metals under physiological conditions, because solubility of cobalt compounds is highly influenced by pH, redox conditions, and the presence of organic species. NTP states, “The metals and poorly soluble compounds tended to be less bioaccessible in neutral biological fluids, which is consistent with the pH dependence for releasing cobalt ions in solution” (NTP 2016b). Therefore, water solubility should not be the measure by which to classify cobalt compounds. OEHHA’s categorization of toxicity and carcinogenic potential of cobalt compounds should be amended to be consistent with the current state of the science.

Response to ToxStrategies Comment 2:

As noted in our Response to Comment #1, OEHHA believes that both water solubility and lung fluid bioaccessibility (i.e., lysosomal fluid) are important factors in determining which IUR best represents a specific cobalt compound.

OEHHA presents the categorization of cobalt compounds on page 1 (Section II) of the cobalt TSD, "Insoluble/poorly soluble cobalt compounds are defined here as having a water solubility of <100mg/L at 20C and would use the IUR of 7.8×10^{-3} $(\mu g/m^3)^{-1}$ for risk assessment" and, "Cobalt compounds that have a water solubility of >100 mg/L at 20C are considered water-soluble and would use the IUR of 8.0×10^{-4} $(\mu g\ Co/m^3)^{-1}$."

In general, OEHHA has observed that water soluble cobalt compounds are salts that have a water solubility considerably greater than 100 mg/L. The most common soluble cobalt compounds used in commerce are presented in Table 1 of the OEHHA cobalt TSD. Insoluble cobalt compounds generally had water solubilities considerably less than 100 mg/L. Below are a few of the water and lysosomal fluid solubilities of cobalt metal and compounds:

Solubilities of some cobalt compounds (NTP, 2016)

Molecular Formula	Form of Cobalt (Metal or Cobalt Compound)	Water solubility g/100 cc (mg/L)	% Solubility in lysosomal fluid
Co	Cobalt metal particles/dust	0.00029 (2.9)	100
CoO	Oxide (II)	0.00049 (4.9)	92.4
Co_3O_4	Oxide (II,III)	0.00016 (1.6)	2-50%
$CoSO_4$	Sulfate (heptahydrate)	60.4 (604,000)	100
$CoCl_2$	Chloride (hexahydrate)	45 (450,000)	100
$Co(C_2H_2O_2)_2$	Acetate (tetrahydrate)	34.8 (348,000)	80
CoN_2O_6	Nitrate (hexahydrate)	67.0 (670,000)	100

For water soluble cobalt compounds, NTP (2016) shows that water solubility is well above 100 mg/L, ranging from 450 to 670 g/L (450,000 to 670,000 mg/L). For the common water insoluble compounds, including cobalt metal and cobalt oxides, water solubility range from 1.6 to 4.9 mg/L. Thus, for some of the more common cobalt compounds water solubility usually fall well below, or well above, 100 mg/L.

However, the major consideration for these compounds is if they are insoluble enough to be largely taken into lung cells in particle form via endocytosis, and then show some release of cobalt ions in lysosomal fluid. Solubility appears to play a role in cobalt-induced lung cell genotoxicity and suggests soluble and insoluble forms of cobalt may have different carcinogenicity potentials (Smith et al. 2014). Categorization based on

water solubility works well because insoluble cobalt metal and compounds appear to be largely internalized by cells as particles.

Thus, the concern by ToxStrategies that water solubility is a poor surrogate for solubility of metals under physiological conditions is not evident with the cobalt compounds most often used commercially (see Table 1, OEHHA Cobalt TSD). However, OEHHA will revise Section II (Health Assessment Values) and Section III (Carcinogenicity) of the OEHHA cobalt TSD to more clearly state up front the importance of water solubility data and lung fluid bioaccessibility data (primarily lysosomal fluid data), and discuss the mechanism of cobalt ion release *in vivo*, which is used to determine the appropriate cobalt IUR for a given cobalt compound. However, virtually all insoluble cobalt compounds appear to have enough solubility in lysosomal fluid to present a cancer risk.

Finally, relying on only lung fluid bioaccessibility would have its drawbacks. As Hillwalker and Anderson (2014) noted in their metal bioaccessibility study, lack of standardization for selecting physiologically-based extraction conditions including residence time, substance mass to biofluid volume ratio, agitation, and biofluid formulation chemistries could make it difficult to compare results between bioaccessibility studies. In addition, the authors showed that minor changes in biofluid formulation have significant effects on bioaccessibility of cobalt compounds and alloys. These limitations also reflect the problems associated with the small amount of lung fluid bioaccessibility data for cobalt compounds. Thus, OEHHA believes that the water solubility of a cobalt compound is currently an important factor for describing the potential fate of inhaled cobalt compounds.

ToxStrategies Comment 3:

1.2 The draft risk assessment document does not contain detailed evaluation of the inhalation bioaccessibility information for cobalt and cobalt compounds.

NTP states, “Evaluation of toxicological and carcinogenic effects of cobalt compounds depends largely on the release of cobalt ions that can either be transported to and taken up at target sites or released within cells from particles” (NTP, 2016). However, the draft OEHHA (2019) risk assessment document does not contain a detailed section on inhalation bioavailability and bioaccessibility of cobalt and cobalt compound, to characterize cobalt ion release. Table 1 in OEHHA (2019) presents only qualitative descriptions of solubility for different cobalt compounds, but no quantitative data on inhalation bioaccessibility are presented. The body of published data for cobalt inhalation bioaccessibility is considerable (see **Table 3** as an example) [data not shown here; refer to the submitted comments]. Table 1 in OEHHA’s draft risk assessment

document needs to be revised to present quantitative data. Additionally, current text in Section 3, Carcinogenicity, needs to be revised and expanded to consider inhalation bioaccessibility information on cobalt and cobalt compounds.

Response to ToxStrategies Comment 3:

OEHHA will revise the first two paragraphs of Section III to clarify which cobalt IUR is to be used for a given cobalt compound based on water solubility data and lung fluid solubility data (if it exists). However, OEHHA believes it is unnecessary to go into great detail with quantitative lung fluid and water solubility data for all cobalt compounds. Keeping the classification information simple, based on water solubility (< or > than 100 mg/L) and some solubility in lysosomal fluids for the insoluble compounds, is adequate for determining which cobalt IUR to use. Nevertheless, to provide greater transparency, OEHHA will add some quantitative solubility data for the cobalt compounds shown in Table 1 of the cobalt TSD, as suggested by ToxStrategies. Table 1 will also indicate the IUR that each cobalt compound should be assigned.

ToxStrategies Comment 4:

1.3 Cobalt in alloys should be considered separately from pure cobalt compounds.

Corrosion- and heat-resistant metal alloys, used by several industries such as aerospace and nuclear, often use metals that include cobalt, nickel, and chromium (ATSDR 2004; IARC 2006). The chromium present in stainless steel forms an impervious oxide layer that limits the solubility of metals in the alloy matrix. Therefore, cobalt in alloys is considered distinctly from pure cobalt compounds, such as cobalt as pure metal and cobalt sulfate, because cobalt in alloys is generally not bioavailable, meaning that cobalt ions are not readily released from the alloy into biological fluids. As shown by Hillwalker and Anderson (2014), cobalt in chromium-enriched alloys is relatively insoluble in lysosomal fluid (**Table 3; Figure 1B**). The solubility of cobalt metal was 30%, whereas the solubility of cobalt in stainless steel and other metal alloys was <0.00027%.

ToxStrategies recently conducted inhalation bioaccessibility testing of cobalt in a baghouse dust sample collected from a metal processing facility in Paramount, California (ToxStrategies 2017). We also evaluated a pure cobalt metal sample for inhalation bioaccessibility. This facility conducts grinding of various metal alloys, and its cobalt emissions are water insoluble and also expected to be insoluble in lung fluids. The objective was to understand whether cobalt in the alloy forms generated from

grinding the metal was bioaccessible/soluble in simulated lung fluids and how that compares to bioaccessibility of the pure cobalt metal.

Bioaccessibility in synthetic lysosomal lung fluid was tested in the laboratory using the experimental methods delineated in Henderson et al. (2014). The baghouse dust and cobalt metal samples were analyzed at Prima Environmental, Inc. Baghouse dust samples were filtered to less than 75 microns using a 200-mesh screen to test particles in the size range most likely to be inhaled. Lysosomal fluids were created using the specifications provided in Table 2 of Henderson et al. (2014). Two incubation time periods (24 hours and 72 hours) were used to understand how bioaccessibility in the lung fluids changes over time as particles are cleared from the lung over days or longer.

Similar to Hillwaker and Anderson (2014), we found that cobalt in alloys had limited bioaccessibility compared to pure cobalt metal (**Table 4; Figure 1B**). With 72-hour incubation in lysosomal fluid, cobalt metal had 40% solubility/bioaccessibility, compared to 2.2% in dust generated from grinding alloys. Cobalt in the alloy form in grinding dust is about 20 times less bioaccessible than cobalt metal in lysosomal fluids. It is clear that an alloy matrix effect is present that limits bioaccessibility of cobalt in an alloy form. Based on this work, the carcinogenic potency of cobalt in the metal dust emitted from the grinding facility was expected to have lower potential for carcinogenicity than pure cobalt metal, and it could be characterized as such. This trend is also observed with other metals in alloys and also in gastric fluids where pH is substantially lower (pH=1.5) compared to lysosomal fluid (pH=4.5) (Henderson et al. 2012, Hillwalker and Anderson 2014, Suh et al. 2019).

Table 4. Inhalation bioaccessibility results for cobalt in samples collected from a metal processing facility in California

Sample	Lysosomal 24-hour	Lysosomal 72-hour
Alloy grinding dust	1.8%	2.2%
Cobalt metal sample	28%	40%

Notably, in the 2016 RoC Monograph, NTP does not specifically address cobalt alloys, because cobalt ions are not released readily from alloys in biological conditions. Hence, consideration of inhalation bioaccessibility information is critical for evaluating cobalt in alloys. We agree with OEHHA that the draft IURs are not applicable to alloys (stated on page 2). However, we also recommend adding further clarification to indicate that all alloy forms are considered for exclusion, not just the cobalt-tungsten hard metal alloys.

Response to ToxStrategies Comment 4:

As indicated in the OEHHA response to Comment #1, we are excluding all cobalt alloys from the cobalt IURs. However, OEHHA would like to point out some possibly misleading assumptions made by ToxStrategies in Comment #4. The cobalt content was only 0.09% or less in the stainless steel tested for bioaccessibility in the study by Hillwalker and Anderson (2014). The lack of measurable cobalt metal release following treatment of steel with lysosomal fluid may be as much a function of the low cobalt content as is the low solubility of the metals in steel. Stopford et al. (2003) observed a cobalt solubility of 26-27% for both pre-sintered and post-sintered cobalt-tungsten alloy following treatment with lysosomal fluid. This is a lower solubility than pure cobalt metal, but this alloy is known to be a more potent carcinogen than cobalt alone. Thus, it is not accurate to suggest, in general, that alloy grinding dust will have a lower potential for carcinogenicity than pure cobalt metal alone, particularly since Comment #4 did not include information on the metal components and their percentages in the alloy grinding dust.

OEHHA welcomes any additional peer-reviewed bioaccessibility data that ToxStrategies or the Cobalt Institute may provide. Summaries of new studies can be included in the OEHHA cobalt TSD if it is published before finalization of the TSD.

ToxStrategies Comment 5:

2. Errors in unit and dosimetric conversions result in inaccurate conclusions regarding the relative carcinogenicity of cobalt sulfate and cobalt metal.

There are errors and unclear statements in OEHHA's draft risk assessment document that create confusion and will likely result in inaccurate air toxics risk assessments when these values are applied. We recommend that OEHHA conduct a comprehensive review of the draft document and provide corrections and revisions of statements that are confusing, and review the NTP (1998) bioassay for cobalt sulfate heptahydrate in detail to better characterize the dose. Specific examples are provided below.

2.1 The conversion calculations for cobalt concentrations from cobalt sulfate heptahydrate concentrations are in error.

It is clear in the NTP (1998) cobalt sulfate heptahydrate study that doses are presented as cobalt sulfate heptahydrate. However, OEHHA converted doses to cobalt ion using the mass of cobalt sulfate, without the waters of hydration. As a result, the molecular weight of cobalt sulfate heptahydrate is underestimated, as is the carcinogenicity, because the mass of cobalt administered is overestimated. OEHHA states that the

conversion was done to compare the NTP (1998) cobalt sulfate heptahydrate data to the NTP (2014) cobalt metal data:

> To compare cancer potencies of the two cobalt forms, the exposure levels for the studies were calculated based on cobalt content alone (Behl et al., 2015). Thus, chamber concentrations of 0, 0.3, 1.0 and 3.0 mg/m^3 cobalt sulfate (CoSO4) corresponds to 0, 0.11, 0.38 and 1.14 mg/m^3 Co, respectively." (page 43, OEHHA 2019)

However, the doses consisted of cobalt sulfate heptahydrate, not cobalt sulfate. This conversion is based on the ratio derived by dividing the molecular weight of cobalt into the molecular weight of cobalt sulfate (58.9 g/mol Co ÷ 154.996 g/mol CoSO4 = 0.38). In Behl et al. (2015) and NTP (1998), the authors indicate that cobalt exposures in the aerosol were primarily in the form of cobalt sulfate **hexa**hydrate to add further confusion to these comparisons:

> Exposure concentrations of cobalt sulfate heptahydrate in this study are expressed as mg cobalt sulfate/m^3; however, it was determined that each mole of aerosol in the exposure chambers **contained an approximate 1:1:6 molar ratio of cobalt:sulfate:water, indicating that exposures were primarily to cobalt sulfate hexahydrate**. [emphasis added] (page 196, Behl et al. 2015)

> The stability of aerosol concentrations in the 0.3 and 3.0 mg/m^3 chambers was monitored by analyzing samples collected on Gelman A/E glass fibers using a calibrated flow sampler. X-ray diffraction analyses were performed by a Philips 3600 diffraction unit with Cu Ka radiation. **Results indicated that cobalt sulfate hexahydrate was the primary species delivered to the chambers**." [Emphasis added] (page 215, NTP 1998)

It is apparent that OEHHA used the conversion calculations from Behl et al. (2015) without considering the cobalt form as described above. We recognize that Behl et al. (2015) also made this error. Perhaps additional confusion was created because the discussion of the predominant form of cobalt sulfate was brief in NTP (1998), and the heptahydrate form was indicated in the title and discussed throughout the report, although hexahydrate seems to have been the administered form.

Regardless, the conversion calculation should not have been based on cobalt sulfate, rather the mass of heptahydrate should have been included. Based on the ratio derived by dividing the molecular weight of cobalt into the molecular weight of cobalt sulfate heptahydrate (58.9 g/mol Co ÷ 281.1 g/mol $CoSO_4$•7H2O = 0.2095), the corrected cobalt content based on the chamber concentrations of 0, 0.3, 1.0, and 3.0 mg/m^3

cobalt sulfate heptahydrate are 0, 0.063, 0.21, and 0.63 mg/m^3 cobalt. These values should be used in the comparison, not the values used in the current draft.

OEHHA used the same approach to normalize the cobalt sulfate heptahydrate cancer slope factor (CSF) to the content of cobalt. A ratio derived by dividing the molecular weight of cobalt into the molecular weight of cobalt sulfate heptahydrate (58.9 g/mol Co ÷ 281.1 g/mol $CoSO_4$•7H2O = 0.2095) was multiplied by a human CSF of 13.41 (mg/kg-day)-1 from cobalt sulfate heptahydrate ($CoSO_4$•7H2O) to calculate a CSF of 2.8 (mg/kg-day)$^{-1}$.

In addition to the conversion of cobalt content, as discussed below, the concentration in air is not the determinant of target-tissue dose to the lung, and a molecular weight conversion, even if done correctly, is inadequate to compare airborne particulate cobalt metal and cobalt sulfate heptahydrate potencies. See Comment 3 for the comprehensive discussion.

Response to ToxStrategies Comment 5:

The NTP (1998) study does indicate that the primary species delivered to the chambers was the hexahydrate on Page 215 of the Methodology Section and in Appendix F. This was determined by X-ray diffraction analysis of samples from the 0.3 and 3.0 mg/m^3 chambers. NTP notes that cobalt heptahydrate dehydrates to the hexahydrate at 41.5ºC, but there is no indication that NTP applied heat during the generation of the hydrated cobalt sulfate aerosol. The generation of the aerosol for rodent exposure involved nebulization of a solution of cobalt sulfate heptahydrate in deionized water. Shear forces broke the stream into droplets that were evaporated, leaving dry particles of the cobalt compound. The aerosol generation and exposure system included primary and secondary compressed-air nebulizers. NTP does not explain why cobalt sulfate hexahydrate, rather than cobalt sulfate heptahydrate, was generated. However, it appears the dehydration/nebulization method removed a water molecule from the heptahydrate form. Under normal environmental conditions, it would be assumed that exposures to hydrated cobalt sulfate will be to the heptahydrate form.

In Section IV of the cobalt TSD, OEHHA summarized the findings of Behl et al. (2015) in which the cobalt sulfate carcinogenicity results (in mg $CoSO_4$ / m^3), without the waters of hydration, were compared to the cobalt metal carcinogenicity results (in mg Co / m^3). OEHHA agrees with ToxStrategies this may not be the most appropriate way to make the comparison if release of the cobalt ion is suspected to be the primary factor for cancer risk. OEHHA will make a comparison of the two cobalt studies based on only cobalt content (as Co) alone. Thus, the cobalt sulfate “hexahydrate” concentrations of

0.3, 1.0 and 3.0 mg/m^3 are converted to Co equivalents of 0.067, 0.22, and 0.67 mg Co/m^3 (58.9 Co / 263.1 $CoSO_4 \cdot 6H_2O$ = 0.223).

In the final calculation of the CSF, we normalize hydrated cobalt sulfate CSF to the content of cobalt. Rather than use the heptahydrate form, as we did in the draft document, we will use the hexahydrate form to derive the CSF. This change results in the CSF adjusted up to 3.0 $(mg/kg\text{-}day)^{-1}$ based on the hexahydrate form, compared to 2.8 $(mg/kg\text{-}day)^{-1}$ when based on the heptahydrate form.

ToxStrategies Comment 6:

2.2 OEHHA compares inhalation exposures between rodents and humans without using a well-established extrapolation method, or whether the extremely high exposures of animal bioassays are environmentally relevant.

OEHHA (2019), notes that:

> The mean cobalt levels of 0.06 to 0.10 mg/m^3 the workers were exposed to were below the lowest cobalt sulfate heptahydrate concentration (0.3 mg/m^3) used in the NTP (1998a) rodent studies - a concentration that did not result in a statistically significant increase at the p = 0.05 level in tumor incidence in the animals by pairwise comparison.

It is not appropriate to simply compare airborne exposure concentrations of particulates between rodents and humans. USEPA provides guidance for such extrapolations (USEPA 1994).

The more relevant comparison of airborne concentrations is that among workers with average exposures of 60,000 to 100,000 ng/m^3 (0.06 to 0.10 mg/m^3) to concentrations in California ambient air. For example, the average concentration of cobalt in the South Coast Air Quality Management District (SCAQMD) ranges from only 0.2 to 0.79 ng/m^3 in the Multiple Air Toxics Exposure Study in the South Basin (MATES IV, SCAQMD 2015). Thus, among workers with exposure concentrations approximately 100,000-times higher than ambient air, no increased risk was observed. We recognize that there are differences in extrapolating results between workers and non-working populations. However, that extrapolation certainly is more noteworthy than comparison with animal data.

Response to ToxStrategies Comment 6:

The comparison of worker cobalt exposures with the rodent exposures was a general comparison, not an extrapolation. OEHHA has revised the document to note this is a direct comparison without adjustment parameters such as inhalation rate and body weight.

In the second part of the comment, the Commenter made a comparison between the highest cobalt exposure of the workers in the Sauni et al. (2017) study and mean levels measured by South Coast AQMD in urban areas of the Los Angeles basin. The study did not describe how many workers were exposed to the highest levels of cobalt, but it could be only a fraction of the 995 workers that participated in the study. The Commenter observed that the workers were exposed to 100,000 times higher cobalt levels than the population in the Los Angeles air basin and still did not experience an increase in cancer cases. In addition to a "healthy worker affect" that ToxStrategies alluded to in their comment, other differences that should be noted include exposure duration. The workers were exposed to cobalt 8 hrs/day, 5 days/week, whereas the Los Angeles population is essentially continuously exposed. The workers were also exposed to cobalt for as little as one year, whereas a significant portion of the population of the Los Angeles basin are exposed for their lifetime. The cobalt worker study by Sauni et al., did not provide a mean worker exposure duration estimate, other than to state that a worker exposure of one year or more was required to be included in the study. The workers also had available to them respiratory protection equipment, but no estimate was provided in the study as to how often this protection was used by the workers. Thus, a comparison of a cohort of cobalt workers to a major urban population is not as strong a comparison as suggested.

ToxStrategies Comment 7:

[OEHHA notes Part 2.3 of the comments by ToxStrategies was missing. It's likely the comment letter did not contain a Part 2.3]

2.4 OEHHA should consider whether the mode of action for chemical carcinogenesis which resulted in rodent tumors is relevant at environmental exposure levels

Further, OEHHA should consider whether the mode of action for tumor formation in rodents in the NTP studies is relevant to environmental exposures. The mechanistic data provided in the NTP (2014) study for cobalt metal, as well as the data discussed in the OEHHA draft guidance, generally support a finding that tumor formation in the lung

is secondary to tissue damage induced by extreme exposures that exceed the maximum tolerated dose in some cases, resulting in oxidative stress and oxidative DNA damage. This is also the finding of Suh et al. (2016). It is highly questionable whether this mode of action exists for environmental exposures to cobalt, which occur at levels that are many orders of magnitude lower. Further, the occupational epidemiology data, as cited by OEHHA, do not indicate that an increased risk of cancer exists in humans at exposure concentrations that are approximately 100,000 times higher than environmental exposures.

OEHHA should further consider the text on page 42, wherein it is stated:

> The cancer hazard of cobalt inhalation was assessed by NTP in separate chronic rodent studies of the water-soluble cobalt compound, cobalt sulfate heptahydrate (NTP, 1998a), and cobalt metal (NTP, 2014a) in male and female rats and mice. Based on the results of these NTP studies, cobalt exhibits carcinogenicity in multiple species, which reflects the greatest potential to induce tumors in other species including humans (Tennant and Spalding, 1996; NTP, 2014a; Behl *et al.*, 2015).

It is certainly not surprising that doses of cobalt, in highly bioaccessible and bioavailable forms, that are sufficiently high to induce oxidative stress and oxidative DNA damage, will cause lung tumors in multiple species in a bioassay. However, the critical question is whether there is the potential for carcinogenicity at relevant human exposure levels and to the forms of cobalt to which people are exposed in ambient air. OEHHA should address this issue. The tumors induced in the bioassay are unlikely to be relevant to environmental human exposures based on both the delivered dose to the lung and the forms of cobalt that exist environmentally.

Application of OEHHA's draft cancer risk assessment, assuming linear extrapolation to the very high exposures that caused cancer in rodents to very low exposure range in ambient air, can have significant implications for environmental risk assessment. As an example, lifetime exposures to cobalt in the metal and insoluble forms, using OEHHA's draft risk assessment and the upper end of the average exposures measured in ambient air, results in a cancer risk of 6 in one million ($0.00079\ \mu g/m^3 \times 7.8 \times 10^{-3}\ [\mu g/m^3]^{-1}$), which exceeds the *de minimus* risk level of 1 in one million. As is evident in this example, significant regulatory actions may result from OEHHA's risk assessment of cobalt metal, and it is vital to the regulated industry and to the public interest, that the forms of cobalt be characterized correctly and that the best scientific methods be used to calculate carcinogenic potency.

Response to ToxStrategies Comment 7:

OEHHA cancer risk assessment policy (OEHHA, 2009) outlines the use of a linear non-threshold dose-response relationship to extrapolate cancer risk from the higher doses used in animal studies to the lower doses encountered by environmentally exposed human populations unless data indicating otherwise exist. In this case, there are no data indicating that a linear non-threshold dose-response relationship should not be used to develop cancer IURs for cobalt and cobalt compounds. As explained in the OEHHA (2005) Cancer Potency Factor TSD, "The procedures used to extrapolate low-dose human cancer risk from animal carcinogenicity data generally assume that most agents that cause cancer also damage DNA, and that the quantal type of biological response characteristic of mutagenesis is associated with a linear non-threshold dose-response relationship. The US Environmental Protection Agency (US EPA) states that the risk assessments made with this model should be regarded as conservative, representing the most plausible upper limit for the risk".

It is unknown if intracellular cobalt levels must reach a "threshold" upon which glutathione (GSH) and other oxidant scavenging peptides/proteins are overwhelmed and oxidative DNA damage then occurs. Additionally it is not clear if this is the only potential mechanism by which cobalt causes genotoxicity, mutagenicity and cancer. Some researchers have observed reduced DNA repair in *in vitro* studies with cobalt exposure, seemingly unrelated to oxidative damage (Kumar et al., 2017). Thus, OEHHA employs a linear non-threshold dose-response relationship in order to extrapolate to lower exposure.

Nickel and chromium are other metals that cause intracellular oxidative stress that may be related to their carcinogenic action (Valko et al., 2005). OEHHA has developed cancer IUR values for these metals as well. Generation of oxygen radicals may also be involved in the carcinogenesis of mercury, cadmium and arsenic. OEHHA has also derived IURs for these metals and metalloids. Thus, cobalt is not the first oxidant-generating metal for which an IUR has been developed.

ToxStrategies suggests Californians in urban settings may be exposed to concentrations of cobalt (as total suspended particulate, or TSP) in the upper mean range of 0.79 ng/m^3, resulting in a cancer risk of 6 in a million (with use of the proposed cobalt IUR). The Hot Spots program under which the cobalt IURs were developed is meant to protect homes and neighborhoods from nearby industries emitting pollutants. It is possible that the upper mean range is a result of air monitors being situated near facilities that emit cobalt. Therefore, the derivation of cobalt IURs is important for

protecting the health of Californians As noted above, the IURs for cobalt do not include metal alloy particles that have cobalt as a component.

ToxStrategies Comment 8:

2.5 The discussion of solubility requires revision.

If OEHHA does not revise the discussion of solubility to be based on bioaccessibility, there is a high likelihood that the IUR for insoluble cobalt will be misused. Forms of cobalt that are insoluble in biological lung fluids should be treated differently from cobalt metal. For example,

- On Page 1, OEHHA states:

  "Insoluble/poorly soluble cobalt compounds are defined here as having a water solubility of ≤100 mg/L at 20°C and would use the IUR of 7.8 X 10^{-3} $(\mu g/m^3)^{-1}$ for risk assessment. This definition of water solubility has been used by other organizations (MAK 2007, USP, 2015)."

First, these two reference citations do not support the use of water solubility for risk assessment. USP (2015) is a pharmacopeia defining solubility, but it is not directly applicable for use in risk assessment. Additionally, water solubility is not specified; rather, solubility is indicated in varying degrees (i.e., very slightly soluble, slightly soluble, sparingly soluble, soluble, freely soluble, and very soluble) (USP, 2015). In MAK (2007), cobalt solubility in serum is presented alongside cobalt solubility in water. It is also stated that "in the case of cobalt metal in powder form, cobalt(II) oxide and cobalt(III) oxide hydrate, a higher solubility was found in blood serum when compared with that in water" (MAK 2007). MAK recognizes the important difference between water solubility and solubility in biological fluids.

Since the release of MAK (2007), NTP published its RoC Monograph on cobalt and cobalt compounds (NTP, 2016b). In the Monograph, detailed discussions of cobalt inhalation bioaccessibility are presented. It is clear that, while cobalt metal powder is poorly soluble in water, it is soluble in all physiologically relevant fluids (NTP, 2016b). Given these factors and as described in Section 3, the rationale for using water solubility to categorize cobalt compounds should be revised and clarified.

Response to ToxStrategies Comment 8:

ToxStrategies did not include the Pharmacopeia (USP, 2015) definition of water solubility/insolubility in their comment. USP defines "practically insoluble or insoluble"

as ≥10,000 mass parts solvent required to dissolve 1 mass part of solute. This is equivalent to ≥100 mg/L (1g solute / 10,000 g solvent is equivalent to 1g /10,000 ml, which is equivalent to 100 mg/L). The intent by OEHHA for including the USP information was simply for support of a quantifiable demarcation for water solubility and insolubility.

Regarding the MAK reference (MAK, 2007), it states that, "For pragmatic reasons, cobalt compounds are divided into two groups, those soluble in water at levels of 0.1 g/L, and those poorly soluble in water at levels below 0.1 g/L." These pragmatic reasons are likely the same as those stated in OEHHAs response to Comment #1: Water solubility is a very common measure of the physical property of a compound, whereas interstitial and alveolar fluid solubility data are limited. Lack of standardization for physiologically based extraction conditions is also a problem. OEHHA believes that categorizing cobalt compounds primarily by water solubility is the main factor in deciding which cobalt IUR applies because it aligns well with what form, particle or ion, cobalt takes when reaching pulmonary epithelial cells.

The higher solubility of cobalt metal in serum (compared to water, alveolar and interstitial fluid solubility) is not surprising, considering cobalt is an essential trace element that likely requires transport systems in the bloodstream. In addition, cobalt metal does appear to be more soluble in alveolar and interstitial fluid compared to pure water:

Cobalt metal powder

Water solubility

Kyono et al., 1992 (ultrafine, MMAD not defined)	1.1 mg/L
NTP, 2016 (MMAD not defined)	2.9 mg/L

Alveolar/Interstitial fluid solubility

Stopford et al., 2003 (7.20 µm mean size)	4-4.8% (800-960 mg/L)

It is unclear why Stopford et al. found much greater solubility of cobalt metal in alveolar and interstitial fluids compared to pure water solubility; no discussion of this difference in solubility was discuss in their report, and they did not determine solubility in pure water themselves for comparison. Different test methodologies and different particle sizes are likely factors for some of the solubility differences between studies. However, as noted in the Response to ToxStrategies Comments #1, the most important physiological factor for carcinogenicity is whether the inhaled cobalt compound will be taken up by lungs cells in particle form, and then solubilized in lysosomes by acidic

lysosomal fluid. In this regard, studies show cobalt metal particles are taken up by lung cells and dissolve in the lysosomes.

Not specifically stated in the comment is that cobalt metal powder is 100% soluble in lysosomal fluid (Stopford et al., 2003). Solubility in lysosomal fluid is what determines if a water-insoluble cobalt compound should be considered a carcinogen with an IUR based on cobalt metal.

As requested by ToxStrategies, OEHHA will revise Section III of the OEHHA Cobalt TSD, as needed, to more clearly state the rationale for using water solubility and lysosomal solubility to categorize cobalt compounds for cancer potency.

ToxStrategies Comment 9:

2.6 OEHHA should compare the carcinogenicity of cobalt sulfate heptahydrate and cobalt metal using equivalent administered doses.

On Page 43, OEHHA's discussion in the first full paragraph is confusing. First, cobalt sulfate concentrations were converted to "cobalt contents" for comparison with the NTP (2014) cobalt metal study concentrations. This totally ignores the property of the exposure material, including the size of the administered particle. At the end of the paragraph, it is stated that "cobalt metal appears to be more effective than cobalt sulfate at inducing lung tumors." If it is indeed appropriate to compare the cobalt contents between the two forms, then the carcinogenic potential should be identical. The fact that the two forms appear to have different potencies based on applied dose is evidence that physical properties affecting dosimetry may be important. In this regard, Suh et al. (2016) converted the two forms of cobalt to human equivalent concentrations (HECs) using the EPA (1994) method and found the carcinogenicity to be similar (see Figure 3, reproduced here as **Figure 2**).

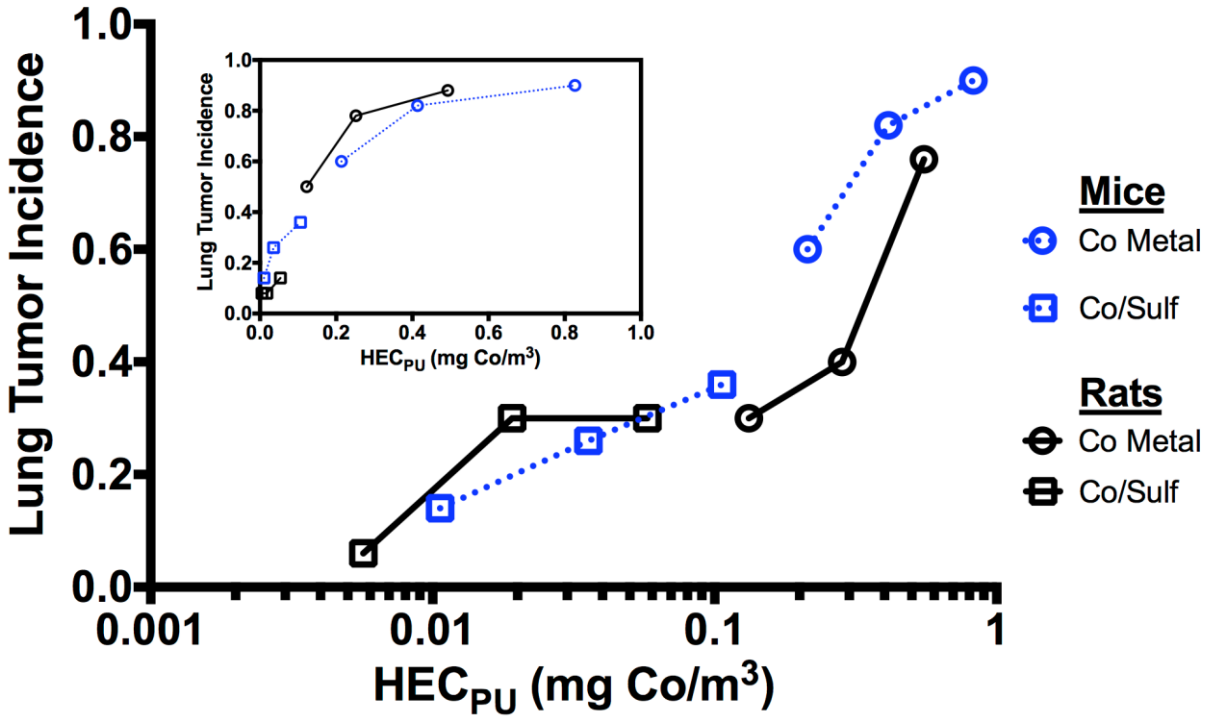

Figure 2. Replicated from Figure 3 of Suh et al. (2016).

The figure above provides lung tumor incidence data in rats and mice from the NTP cobalt metal and cobalt sulfate heptahydrate 2-year cancer bioassays. For the latter, particle size characterization data (e.g., mass median aerodynamic diameter [MMAD] and geometric standard deviation [GSD] of particle sizes) for cobalt sulfate heptahydrate were used assuming that water was included in the mass. The HEC was then adjusted to the cobalt fraction of cobalt sulfate heptahydrate. The main plot shows the data for male and female rats and mice on a log x-axis. The insert shows the data on a linear scale.

Response to ToxStrategies Comment 9:

As stated in the response to Comment #5, OEHHA will revise the discussion of the comparison of cancer potency between cobalt metal and cobalt sulfate heptahydrate, by comparing only the cobalt content of the hydrated cobalt sulfate (i.e., without the sulfate and waters of hydration) with that of cobalt metal.

The method OEHHA used to extrapolate from rodents to humans assumes 100% absorption of inhaled particles in both rodents and humans. The inhaled dose in rodents was determined using equations that determine the average inhalation rate in

rats (OEHHA, 2018) and mice (Anderson, 1983), based on average body weights of the rodents during the 2-year exposure studies. It is correct that MMAD of the particles was not part of these equations. However, particle size differences were minor between the cobalt metal and cobalt sulfate heptahydrate studies. The cobalt metal MMAD was between 1.4 and 2.0 µm (± 1.6-1.9 GSD), depending on the exposure concentration. For cobalt sulfate heptahydrate, the MMADs for the exposure concentrations varied from 1 to 3 µm.

OEHHA employs US EPA's Benchmark Dose (BMD) software to determine cancer slope factors (CSFs) for each tumor type in rats and mice. ToxStrategies also uses this software to derive CSFs, although there are several differences in how this software is used by OEHHA and ToxStrategies. For extrapolation from rodents to humans, OEHHA converts the rodent CSFs to human equivalents using body weight ($BW^{3/4}$) scaling:

$$CSF_{(human)} = CSF_{(rodent)} \times (BW_{(human)} / BW_{(animal)})^{1/4}$$

OEHHA uses this method for CSF derivation due to systemic distribution of cobalt to other organs in the rat that resulted in adrenal medulla tumors, pancreatic islet cell tumors and leukemia. Using the ¾ power body weight scaling follows OEHHA IUR derivation methodology, as described in the Cancer TSD, which does not distinguish between systemic and "point of contact" carcinogens (OEHHA, 2009).

ToxStrategies used US EPA's Regionally Deposited Dose Ratio (RDDR) software to adjust the cobalt concentrations in exposed rodents to human equivalent concentrations (HECs) for determining CSFs based on lung tumors alone. The ratio adjusts for differences in lung surface area, respiratory rate, and fractional deposition. Fractional deposition is determined in three regions of the lung, the upper respiratory, tracheobronchial, and the pulmonary regions. This method includes particle size in deposition calculations. ToxStrategies determined the fractional deposition of the pulmonary region but not the tracheabronchial region. This could result in an underestimation of absorbed dose, since lung tumors may originate from bronchiolar tissue as well. ToxStrategies then applied the adjusted doses and NTP tumor incidence data into the US EPA BMD software to estimate the CPFs.

ToxStrategies suggests that a line could be drawn through the combined cobalt metal and cobalt sulfate heptahydrate data points of the log-dose graph in Figure 2 to suggest a monotonic dose-response is produced. However, if lines were drawn through the cobalt metal and cobalt sulfate heptahydrate data separately, the cobalt metal slopes are steeper compared to the cobalt sulfate slopes. The steeper slopes would indicate

that cobalt metal is a more potent carcinogen than cobalt sulfate heptahydrate. This is what the OEHHA-derived IUR values show – that cobalt metal is nearly 10-fold more potent a carcinogen than cobalt sulfate heptahydrate.

Differences in cellular uptake between soluble and insoluble forms of cobalt have been proposed as a reason for differences in cancer potency. It has been shown that cobalt nanoparticles *in vitro* interact with proteins on the surface of cells and are readily taken up by those cells (Ponti et al., 2009; Colognato et al., 2008). This resulted in a 50- to 140-fold greater cellular uptake and intracellular release of cobalt ion from insoluble cobalt (i.e., cobalt(II) oxide) vs. uptake of extracellular ions from a soluble cobalt compound (cobalt chloride). We go on to state on Page 18 of the Cobalt TSD that, "Further research suggests internalized cobalt metal nano- and micro-particles diffuse to subcellular organelles and release cobalt ion in millimolar concentrations in nuclei and mitochondria (Sabbioni et al., 2014a,b)." On page 28 of the Cobalt TSD we summarize that, "…*in vitro* genotoxicity studies by Smith et al., (2014) led to the conclusion that solubility appears to play a role in cobalt-induced lung cell genotoxicity and suggests soluble and insoluble forms of cobalt may have different carcinogenicity potentials."

ToxStrategies Comment 10:

3. Refinements to the Cobalt Risk Assessment Methods Used by OEHHA

The Suh et al. (2016) paper, "Inhalation cancer risk assessment of cobalt metal," published in *Regulatory Toxicology and Pharmacology*, is highly relevant to OEHHA's IURs, yet it is cited only once, and not in the cancer risk assessment section.

On Page 20, OEHHA cites Suh et al. (2016) for the following statement:

> Thus, the equivocal increased cancer risk noted by Tuchsen et al. may be related to the lack of significant *in vivo* release of cobalt ions from cobalt aluminate spinel (Suh et al. 2016).

In fact, Suh et al. does not make this statement, but we don't disagree with the statement. Aside from that, we are puzzled because OEHHA does not discuss the study in Section V, Quantitative Cancer Risk Assessment, where it is clearly most relevant. We recommend that OEHHA review the Suh study and revise the assessment.

We offer several specific refinements to improve the risk assessment methods of the OEHHA draft. As authors of the Suh et al. (2016) publication of a cobalt metal IUR, our comments focus on a comparison of the methods used by OEHHA as compared to our paper. **Table 5** compares selected IUR values derived by OEHHA with those published

in Suh et al. (2016). Specifically, we show comparisons for male rats and mice, which resulted in the highest IURs for cobalt metal, as derived in OEHHA (2019). Overall, the recommended IURs determined by OEHHA and Suh et al. (2016) differ by 2.6-fold (IUR values of 7.8E-3 vs. 3.0E-3). As will be discussed, these values were derived using different approaches.

Table 5. Comparison of selected IUR values between OEHHA (2019) and Suh et al. (2016)

Endpoint	OEHHA (2019) Human CSF $(mg/kg\text{-}day)^{-1}$	OEHHA (2019) Human IUR $(\mu g/m^3)^{-1}$	Suh et al. (2016) Human IUR $(\mu g/m^3)^{-1}$	Suh et al. (2016)[b] Human IUR (ALT) $(\mu g/m^3)^{-1}$
Male rat A/B tumors	12.91	3.7E-3	5.8E-3	4.5E-3
Male rat pheochromocytomas	9.51	2.7E-3	6.3E-4	NC
Male rat pancreatic	1.71	4.9E-4	1.1E-4	NC
Combo: Male rat (all three)	22.17	6.3E-3	NC	NC
Combo: Male rat (lung & pancreas)[a]	14.1	4.0E-3	NC	NC
Male mouse A/B tumors	27.49	7.9E-3	5.7E-3	3.1E-3
Final proposed value	27	7.8E-3		3.0E-3[c]

NC = not conducted
Shaded row for male mouse tumors was selected by OEHHA as the basis for an IUR
a Analysis not conducted by OEHHA, but shown here for comparison (derived by ToxStrategies using OEHHA method)
b Analysis conducted using custom benchmark response (BMR) approach (see Table 4 in Suh et al. 2016)
c Final value was based on 3.4E-3 average of IURs for male and female rats and mice (rounded to one significant figure; see Table 4 in Suh et al. (2016))

Response to ToxStrategies Comment 10:

OEHHA relied on methodology that has been used to derive cancer potency values for our various programs, including the Proposition 65 program (OEHHA, 2009). We feel the methods are health protective and appropriate. OEHHA Cancer IUR derivation documents generally do not include a discussion of risk assessment methods employed by other groups, unless they contain new toxicology data.

Table 5 shows that the IURs derived in Suh et al. (2016) and by OEHHA are remarkably close, considering the different methods used to derive the values at nearly every step

of the risk assessments. However, OEHHA does not agree that the BMD alternate (ALT) method used in Suh et al. (2016) is the most appropriate. A response regarding the ALT method is presented below in ***Response to ToxStrategies Comment #15***.

ToxStrategies Comment 11:

3.1 OEHHA did not follow its own guidance on benchmark response (BMR) selection.

On page 50, OEHHA states, "For large datasets such as those by NTP, the BMD recommended by OEHHA (2008) is the 95% lower confidence bound on the effective dose producing 5% response (BMDL05)."

The citation supporting the 5% BMR is OEHHA (2008), which is a document focusing on noncancer effects:

> OEHHA. 2008. Air Toxics Hot Spots Program risk assessment guidelines. Technical support document for the derivation of noncancer reference exposure levels. California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Oakland, CA. Online at: http://www.oehha.ca.gov/air/hot_spots/rels_dec2008.html.

It is unclear why OEHHA did not cite the more recent 2009 guidance on developing cancer potency factors:

> OEHHA. 2009. Technical support document for cancer potency. California Environmental Protection Agency, Office of Environmental Health Hazard Assessment.

In the (2009) guidance, OEHHA states:

> The benchmark chosen is a point at the low end of the observable dose-response curve. Usually a dose at which the incidence of the tumor is 10% is chosen for animal studies, although lower effect levels may be appropriate for large epidemiological data sets. Because real experimental data include variability in the response of individual subjects, and measurement errors, likelihood methodology is applied in fitting the data. A lower confidence bound (usually 95%) of the effective dose (LED10), rather than its maximum likelihood estimate (MLE), is used as the point of departure.

Importantly, neither the 5% nor the 10% response rate is near the observable range for the NTP cobalt metal bioassay, because NTP administered only very high doses of cobalt metal. Further, OEHHA did not follow its own guidance by selecting the 5% BMR.

Response to ToxStrategies Comment 11:

OEHHA generally considers the NTP datasets with 50 animals/sex/dose to be a large dataset such that the 95% lower confidence bound on the effective dose producing 5% response is appropriate to use. We state on Page 17 of the OEHHA (2009) guidance, "Whereas the exposed population of an epidemiological study might number in the thousands, a typical animal study might have fifty individuals per exposure group. With this group size any phenomenon with an incidence of less than about 5% is likely to be undetectable." Thus, we use a BMR of 5% (and not lower) in our risk assessment for datasets of this size.

In analyzing the data on lung tumors in male mice, which formed the basis for the cancer potency estimate for cobalt metal, the lowest non-zero dose was considerably greater than the dose associated with a BMR of 5%, the BMD_{05}. In cases such as this, using a BMR higher than 5% yields a BMD closer to the lowest non-zero dose. See the response to comment #15 for a detailed discussion of the approach to selecting a BMR for this data.

ToxStrategies Comment 12:

3.2 OEHHA did not use dosimetric adjustments appropriate for each tumor site, which is inconsistent with USEPA guidance and ignores the importance of variable lung deposition by particle size and species.

USEPA uses the guidance document Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (USEPA 1994) for adjusting inhalation exposures to various regions of the body—depending on the location of the lesion of interest (including tumors). This method takes into account physicochemical characteristics of the test article (e.g., particle diameter), and well as the anatomy of the target species. Overall, USEPA (1994) provides methods for estimating target-tissue dosimetry to the respiratory tract, as well as dosimetry beyond the respiratory tract. Instead, on page 49, OEHHA simply converted the duration-adjusted inhalation concentration to a rodent body burden using inhalation-rate data and bodyweights. This ignores the particle size information, as well as the target-tissue dosimetry.

Response to ToxStrategies Comment 12:

Because there is evidence of systemic distribution following cobalt metal inhalation to induce tumors at non-pulmonary sites in rats, we used body weight ($BW^{3/4}$) scaling to convert to human equivalents. This is a method used by OEHHA for extrapolating from rodents to humans in CPF derivations. As stated in the Cobalt TSD, "Using this interspecies scaling factor is preferred by OEHHA because it is assumed to account not only for pharmacokinetic differences (e.g., breathing rate, metabolism), but also for pharmacodynamic considerations, i.e., tissue responses to chemical exposure (US EPA, 2005). Lifetime body weights for control rats and mice of both sexes were calculated from the NTP (2014) study as described above. The default body weight for humans is 70 kg. The body weight scaling factor assumes that mg/surface area/day is an equivalent dose between species (OEHHA, 2009)."

ToxStrategies Comment 13:

3.3 OEHHA did not use dosimetric adjustments appropriate for each tumor site (i.e., inconsistent with U.S. EPA guidance).

By using the method described in USEPA (1994), exposures to rodents can be converted to human equivalent concentrations (HECs). Following duration and dose adjustment, the tumor data can be modeled in terms of HEC. Suh et al. (2016) modeled effects in the rodent lung, pancreas, and adrenal medulla in terms of HEC. These endpoints required different adjustments, because lung tumors were most likely a site-of-contact effect, whereas the pancreas effects were likely a result of systemic distribution. There is considerable uncertainty regarding the pheochromocytomas in rats, due to their questionable human relevance and evidence for pheochromocytomas arising secondary to lung effects in rodents (see Section 3.4). Together with the issues discussed in Section 3.2, OEHHA has not used standard methods for developing IUR values.

Response to ToxStrategies Comment 13:

Using the US EPA (1994) RDDR to derive a HEC for lung toxicants has been used in the OEHHA Hot Spots program for noncancer Reference Exposure Levels (RELs). However, the US EPA RDDR method is somewhat outdated and a different model, the Multiple Particle Path Dosimetry (MPPD) model is now being promoted as superior for particulate pulmonary toxicant risk assessment (ARA, 2017). ToxStrategies might want to consider using the MPPD model approach for future toxicants. OEHHA has chosen to derive cancer IURs for cobalt metal and cobalt sulfate hepathydrate using body

weight ($BW^{3/4}$) scaling to convert to human equivalents for the reasons described in the above ***Response to ToxStrategies Comment # 12***.

ToxStrategies Comment 14:

3.4 OEHHA failed to consider human relevance for certain rodent tumors.

OEHHA modeled pheochromocytomas in rats both independently and as part of a combined analysis. As will be discussed below, there is evidence that pheochromocytomas arise in inhalation studies where hypoxia is induced either as a consequence of exposure to particulate or lung lesions (including tumors). As stated in the NTP (2014) cobalt metal bioassay:

> The results of several NTP inhalation studies with particulate compounds suggest that there may be an association between the occurrence of benign and malignant alveolar/bronchiolar neoplasms and variably extensive chronic pulmonary nonneoplastic lesions of the lung and significantly increased incidences of hyperplasias and benign and malignant pheochromocytomas of the adrenal medulla in exposed male and female rats.

This relationship can also be surmised by the tumor data. According to Table 8 in OEHHA (2019), the incidence of pheochromocytomas in untreated male rats was 17/46, whereas the incidence of lung tumors was 2/47. This indicates a vast difference in the background incidence in these tumors. Yet, in all the treatment groups, the numbers of male rats with pheochromocytomas were slightly *lower* than those with lung tumors. If the pheochromocytoma tumor responses were independent of lung tumors, one would expect to see more animals with pheochromocytomas, due to systemic exposure to cobalt, than lung tumors among the exposed animals.

NTP (2014) also states:

> Agents that induce adrenal medullary neoplasia tend to be nongenotoxic and seemingly induce carcinogenesis through an indirect mechanism (Strandberg, 1995). In NTP studies, the mechanism(s) responsible for the induction of pheochromocytoma in rats is not understood. However, it is thought that reduced gas exchange induced by extensive space-occupying neoplasms and nonneoplastic lung lesions such as fibrosis and chronic inflammation leads to systemic hypoxemia that chronically stimulates catecholamine secretion from the adrenal medulla. This chronic hypersecretory activity may lead to medullary hyperplasia and neoplasia (Ozaki *et al.*, 2002).

The NTP (2014) report notes that abnormal breathing was observed in rats in shorter-term studies as well as the chronic bioassay, indicating that exposure to cobalt metal particulate induced breathing issues in rats with or without the presence of lung tumors. Thus, there was evidence for treatment-related hypoxia in the NTP cobalt metal study.

Critically, experts in clinical toxicology have concluded that pheochromocytomas in rats "have little or no relevance to human safety" (Greaves 2012). Therefore, it is unnecessary for pheochromocytomas to serve as a basis for any CSF or IUR (alone or in combination) when a more relevant site-of-contact tumor (i.e., lung tumor) is present, and combining the tumors is not appropriate because pheochromocytomas are dependent on lung tumors and other respiratory damage.

Response to ToxStrategies Comment 14:

As noted above, NTP states that the development of pheochromocytomas in inhalation studies are not understood. In addition, NTP states in Behl et al. (2015) that, "Additional studies are needed to investigate whether the adrenal response is related to the presence of these extensive space occupying pulmonary lesions rather than due to a chemical specific response."

Lastly, the NTP Report on Carcinogens (2016) concluded, "Adrenal gland neoplasms can develop because of damage to lungs that causes obstructive sequelae by causing systemic hypoxemia, leading to chronic stimulation of catecholamine release by the adrenal medulla and subsequent neoplastic development (NTP 2014). Since inhalation of cobalt caused lesions in the lung that could cause obstruction (chronic inflammation), it is possible that the adrenal glands are not directly caused by systemic exposure to cobalt, but could be a secondary response to lung damage. However, there is not enough evidence to differentiate between a direct or indirect cause of adrenal gland neoplasms from cobalt exposure."

Due to the lack of confidence for the cause of the rat pheochromocytomas, OEHHA has chosen a health protective approach by assuming that pheochromocytomas arise independently from the lung cancer and noncancer effects. Neither of the NTP cobalt reports suggest that pheochromocytomas in rats "have little or no relevance to human safety", as suggested in Greaves (2012). It would be improper for OEHHA to assume these tumors have no relevance to humans.

A cursory search of NTP technical reports did turn up five carcinogenicity studies, other than cobalt metal and cobalt sulfate heptahydrate, in which inhalation exposure to a chemical resulted in "some" or "clear" evidence of pulmonary tumors, noncancer lung damage and pheochromocytomas in rats. However, there were at least 11 NTP

carcinogenicity studies that showed “some”, “clear” or “positive” evidence of pheochromocytomas resulting from a chemical in feed or administered by gavage in which no pulmonary effects were found. In addition, an inhalation carcinogenicity study of Stoddard Solvent produced some evidence of pheochromocytomas in male rats, but no evidence of lung tumors or lung injury. Therefore, OEHHA cannot ignore the possibility that inhaled cobalt metal and cobalt compounds that are absorbed systemically and reach the adrenal glands could be a direct cause of pheochromocytoma.

The fact that increased lung tumor incidence does not track perfectly with increased pheochromocytoma incidence in rats is not an unusual finding for multi-site carcinogens. The important point is that cobalt metal exposure led to a statistically significant increase in pheochromocytomas in male and female rats at the two highest dose levels, and exhibited a statistically significant positive trend for this tumor type. Cobalt sulfate heptahydrate exposure led to a statistically significant increase in pheochromocytomas in female rats at the highest dose level, and exhibited a statistically significant positive trend for this tumor type.

Regarding the comment about abnormal breathing in the rats, NTP did note that abnormal breathing was observed in some rats. It was not clear from the report which group of rats, and how many, were affected. However, NTP did not find clinical signs of cyanosis in any rats.

ToxStrategies Comment 15:

3.5 OEHHA used model results with large amounts of uncertainty due to extrapolation below the range of observation.

The BMD and BMDL values that OEHHA used for deriving slope factors for lung tumors in rats and mice were highly uncertain due to the BMD and BMDL values being well below the lowest exposure dose in the study. Because OEHHA ultimately derived their IUR based on the male mouse lung tumors, we focus here on those modeling results.

Using OEHHA’s approach of converting inhaled dose to body burden, we were able to replicate several values reported in Table 11 of OEHHA (2019). Although the BMD modeling results in BMDS v2.7 indicated an acceptable p-value for model fit, the BMD5 is well below the range of observation. Dividing the lowest exposure dose (0.26 mg/kg-day) by the BMD5 (0.0145 mg/kg-day) results in extrapolation ~18-fold below the range of observation (note: the BMDL5 is even further below the range of observation at ~23−fold.

We further ran these data in the latest version of BMDS 3.1 (USEPA 2019), which now contains recommendations (and warnings) for model selection, results in recommendations for all models used by OEHHA to be flagged as "Unusable" or "Questionable." All three Multistage cancer models result in "Questionable" due to warnings about (1) "BMD 3x lower than lowest non-zero dose," and (2) "BMDL 10x lower than lowest non-zero dose."

Notably, Suh et al. (2016) modeled the lung tumor data without such extrapolations below the observable range by deriving a custom BMR that would result in the BMD being within the range of observation. This method has been used previously by USEPA wherein the standard BMR of 10% results in BMD/BMDL values far below the range of observation (USEPA 2011). In USEPA's method, the custom BMR is calculated as follows:

$$BMR_{custom} = [P(\text{lowest dose group}) - P(\text{control})] \div [1 - P(\text{control})]$$

Again, using OEHHA's approach of converting inhaled dose to body burden, but using a custom BMR of 78%, returns Multistage models with recommendations of "Viable – Alternate" and $BMDL_{78}$ values of 0.3311 mg/kg-day (notably, the new Bayesian model-averaged BMDL in BMDS v3.1 results in a similar $BMDL_{78}$ of 0.288 mg/kg). The resulting rodent CSF is 2.36 per mg/kg-day (0.78/0.3311), and the human CSF is 14.5 per mg/kg-day. As shown in **Table 6**, OEHHA would have derived an IUR similar to that proposed by Suh et al. (2016) if BMD modeling had been conducted using methods that did not require extrapolation below the range of observation. This suggests that OEHHA's use of BMD/L values well below the range of observation results in an IUR ~2-fold higher than that proposed by Suh et al. (2016). However, we reiterate that OEHHA's method of converting inhaled dose to body burden without considering the methods described in USEPA (1994) is also problematic (see Sections 3.3 and 3.4).

Table 6. Comparison of select IUR values between OEHHA (2019) and Suh et al. (2016)

Endpoint	OEHHA (2019) Human CSF $(mg/kg\text{-}day)^{-1}$	OEHHA (2019) Human IUR $(\mu g/m^3)^{-1}$	Suh et al. (2016) Human IUR $(\mu g/m^3)^{-1}$
Male mouse A/B tumors (BMR=5%)	27.49	7.9E-3	ND
Hypothetical OEHHA analysis[a]: Male mouse A/B tumors (BMR=78%)[b]	14.5	4.2E-3	3.1E-3

[a] Analysis not conducted by OEHHA, but shown here for comparison (derived by ToxStrategies using OEHHA method)

[b] Analysis conducted using custom BMR approach (see Table 4 in Suh et al. 2016)

Response to ToxStrategies Comment 15:

In the cobalt IUR document, lung tumors in male mice results in the highest cancer potency for cobalt metal. In Benchmark Dose Software (BMDS) version 3.1, a BMR of 5% yields a "questionable" BMD and Benchmark Dose Lower Confidence Limit (BMDL) because the BMD_{05} is more than 3 times lower than the lowest non-zero dose, and the BMDL is more than 10 times lower than the lowest non-zero dose.

To address the Comment that the BMD_{05} for male mouse lung tumors is below the observable range, OEHHA will revise the IUR derivation to include a summary of the multistage polynomial model and the application of the exact formula to obtain the BMDL:

The lifetime probability of a tumor at a specific site given exposure to a chemical at dose d is modeled using the multistage polynomial model:

$$p(d) = \beta_0 + (1 - \beta_0)\left(1 - \exp\left[-\left(\beta_1 d + \beta_2 d^2 + \cdots + \beta_j d^j\right)\right]\right)$$

where the background probability of tumor, β_0, is between 0 and 1 and the coefficients β_i, i = 1…j, are positive. The β_i are parameters of the model, which are taken to be constants and are estimated from the data. The parameter β_0 provides the basis for estimating the background lifetime probability of the tumor. The upper 95% confidence limit on the parameter β_1 is often called the cancer potency or cancer slope factor, since for small doses it is the upper bound on the ratio of extra lifetime cancer risk to the average daily dose received.

In order to derive a cancer slope factor, OEHHA fits the multistage polynomial model to cancer dose-response data using maximum likelihood and estimates the cancer slope factor as the upper bound on β_1 using profile likelihood. There are different software programs available that can carry out these calculations. US EPA's Benchmark Dose Software (BMDS)[1] is typically used because it is widely available. While other software calculates the cancer slope factor (upper bound on β_1) directly, BMDS estimates other values that can be used to calculate the cancer slope factor.

BMDS requires the specification of a benchmark response (BMR). In the case of cancer dose-response modeling OEHHA typically sets the BMR (extra risk of a tumor) equal to 5%. The dose associated with this risk is defined as the BMD_{05} and the lower 95% confidence bound on that dose is defined as the $BMDL_{05}$. Instead of calculating an upper bound on β_1 directly, BMDS uses an approximation to calculate the upper bound on β_1 and reports this as the cancer slope factor: BMR/BMDL.

In some cases, the lowest non-zero dose is considerably greater than the BMD_{05}. In such cases, using a BMR higher than 5% yields a BMD closer to the lowest non-zero dose. In these cases, OEHHA uses the following formula for the calculation of the cancer slope factor (upper bound on β_1): CSF = -ln(1-BMR)/BMDL. This conservative estimate is derived by solving for β_1 in the risk equation and inserting the result into the log-likelihood equation for β_1 to use it to profile the BMD and obtain the BMDL. The expression CSF = -ln(1-BMR)/BMDL is constant over different values of the BMR and this approach appropriately accounts for the increased curvature in the dose response relationship at higher doses and BMRs.

As noted by the commenter, in deriving a measure of the cancer response to cobalt metal (per mg/kg-day) from the data on male mice, the BMD_{05} was over 10 times lower than the lowest non-zero dose used in the study. This is because a large fraction of the animals in each treatment group, including the lowest dose group, had lung tumors. Because of this, OEHHA calculated the "animal cancer slope factor (CSF_a)", or the "animal cancer potency", for male mice using the exact formula described above: -ln(1-BMR)/BMDL, at a higher BMR, in this case, 15%. As shown in Table 15-1 below, not only does setting the BMR to 15% result in a viable model from BMDS 3.1, but the choice of BMR has no effect on the value of the animal cancer slope factor when the exact formula is used to calculate the CSF_a. The value of the CSF_a calculated using the exact formula remains unchanged even when the BMR is set to a value larger than 15%.

[1] US EPA Benchmark Dose Software (BMDS) Version 3.1. National Center for Environmental Assessment, US EPA. Available from: https://www.epa.gov/bmds

Table 15-1. Animal cancer slope factor (CSF_a) calculated in BMDS 3.1 using the approximation CSF_a = BMR/BMDL and calculated using the exact formula CSF_a = -ln(1-BMR)/BMDL

BMDS 3.1 output					CSF_a calculated using exact formula -ln(1-BMR)/BMDL
Model	**BMDL**	**CSF_a**	**BMDS "Recommen-dation"**	**BMDS "Recommendation notes"**	
BMR05 1st degree polynomial	0.01122	4.46	Questionable	BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose BMD 10x lower than lowest non-zero dose BMDL 10x lower than lowest non-zero dose	= -ln(1-0.05)/0.01122 = 4.57
BMR10 1st degree polynomial	0.02304	4.34	Questionable	BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose BMDL 10x lower than lowest non-zero dose	= -ln(1-0.10)/0.02304 = 4.57
BMR15 1st degree polynomial	0.03554	4.22	Viable - Recommended	BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose Lowest AIC	= -ln(1-0.15)/0.03554 = 4.57

Figure 15-1 below is the multistage model fit to the male mouse lung tumor data for cobalt metal with a BMR of 15%.

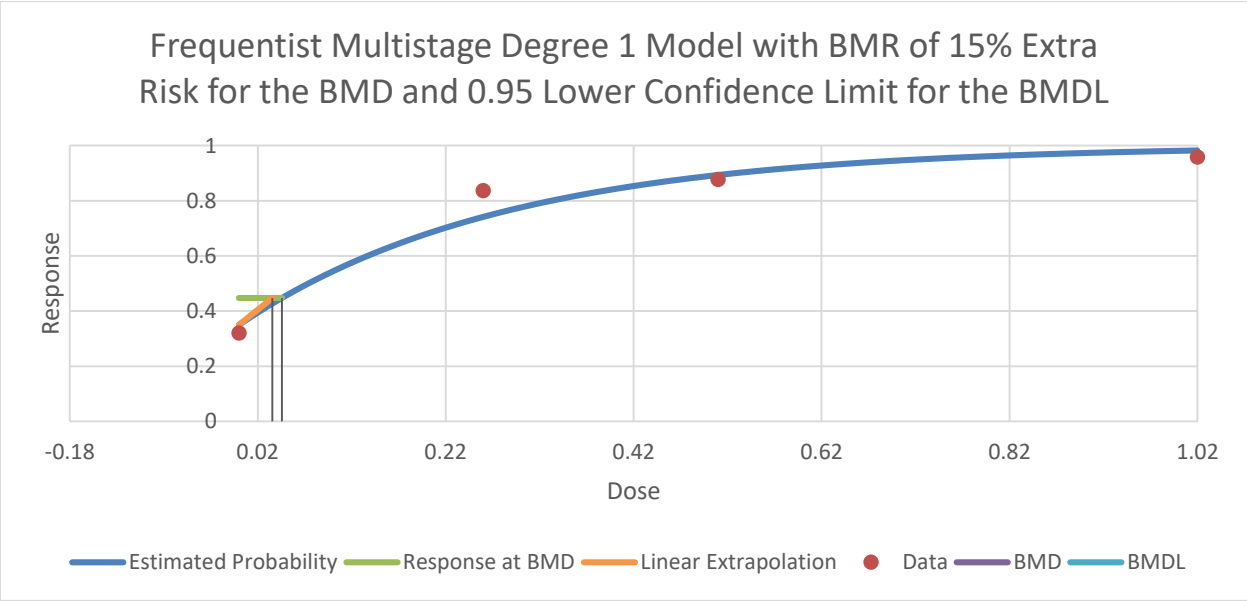

Figure 15-1. Multistage model fit to the male mouse lung tumor data for cobalt metal. The benchmark used is the exposure concentration producing 15% tumor response (BMD) with the 95% lower confidence bound (BMDL) on the BMD.

OEHHA notes that the method of deriving a custom BMR described by the commenter as "used previously by US EPA wherein the standard BMR of 10% results in BMD/BMDL values far below the range of observation (US EPA 2011)," cites an external review draft of the Toxicological Review of vanadium pentoxide. The 2011 draft document has not been finalized and contains a header stating, "DRAFT – DO NOT CITE OR QUOTE," a disclaimer page stating that the document "has not been formally disseminated by EPA" and "does not represent and should not be construed to represent any Agency determination or policy," and, finally, a footer also stating that the document "is a draft for review purposes only and does not constitute Agency policy." Furthermore, the method of deriving a custom BMR is neither discussed nor prescribed in the BMDS 3.1 User Guide, the BMDS Technical Guidance, nor the 2005 Guidelines for Carcinogen Risk Assessment.

Using the BMR custom equation to derive an Alternative (ALT) BMR by ToxStrategies, which raises the BMR to 78% response rate, is unnecessary and not as health protective as OEHHA's approach. A BMD_{78}, as suggested by ToxStrategies, is between the low- and mid-dose groups, and results in a human CSF = 14.55 $(mg/kg\text{-}day)^{-1}$ (Figure 15-2):

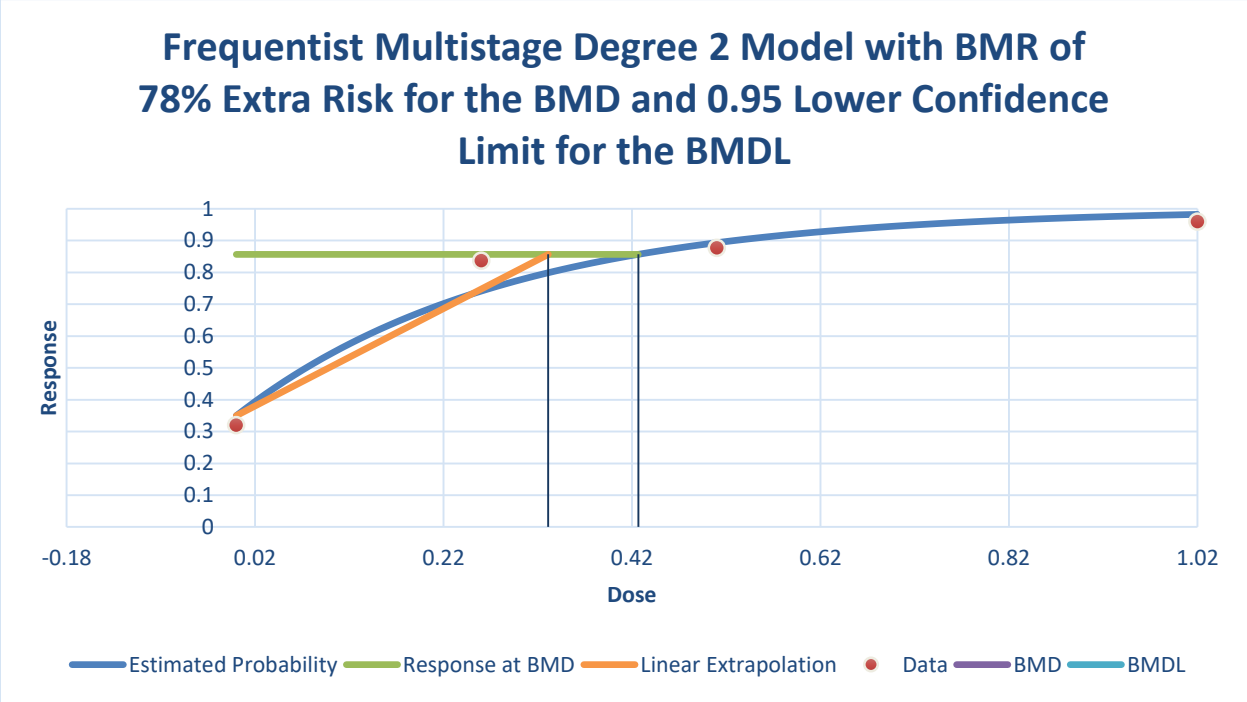

Figure 15-2 Multistage model fit to the male mouse lung tumor data for cobalt metal. The benchmark used is the exposure concentration producing 78% tumor response (BMD) with the 95% lower confidence bound (BMDL) on the BMD.

The ALT approach suggested by ToxStrategies does not take advantage of the dose-response slope between the lowest dose and the control group, where the greatest concern for environmental exposures would exist, and is not considered a health protective approach for cancer risk assessment by OEHHA.

ToxStrategies Comment 16:

3.6 OEHHA's use of the MS_Combo model is inappropriate due to likely interdependence of tumors

OEHHA conducted modeling for the combined tumor incidence in male rats, as well as female rats. We replicated the combined modeling results for male rats using MS_Combo model in BMDS 3.1. While the numbers appear correct, the analysis is flawed, because MS_Combo assumes that the tumors modeled arise independent of one another. In fact, as discussed above, researchers recognize that pheochromocytomas arise secondary to lung tumors. On page 51, OEHHA acknowledges that there is some evidence that pheochromocytomas of the adrenal medulla in rodents might be "dependent on tumor formation in the lungs." More specifically, it is hypothesized that tumor formation and/or particle overload can lead to

hypoxia-related catecholamine secretion from the adrenal medulla and stimulation of medullary hyperplasia that ultimately leads to adrenal pheochromocytomas (NTP 2014; Suh et al. 2016). Notably, medullary hyperplasia was observed in the NTP (2014) cobalt metal study but not the NTP (1998) cobalt sulfate heptahydrate study.

Response to ToxStrategies Comment 16:

For cobalt metal, MS_ Combo was not used to derive the CSF because a single tumor type (i.e., lung tumors) in male mice was used to derive a CSF for cobalt metal. This was the only tumor type observed in exposed male and female mice. For rats, using MS_Combo to combine tumor types, including pheochromocytoma, resulted in a lower CSF compared to the CSF calculated for lung tumors in male mice.

On the other hand, use of MS_Combo was relevant for calculating the highest CSF for cobalt sulfate heptahydrate. Clear evidence of pheochromocytoma was observed in female rats exposed to cobalt sulfate heptahydrate, which was combined with lung tumor incidence data in MS_Combo to derive a CSF for cobalt sulfate heptahydrate.

As explained in ***Response to ToxStrategies Comment 14***, there is not enough evidence to differentiate between a direct or indirect cause of adrenal gland neoplasms from cobalt exposure (NTP, 2016). Additional studies are needed to investigate whether the adrenal response is related to the presence of these extensive space occupying pulmonary lesions rather than due to a chemical specific response (Behl et al., 2015).

Thus, OEHHA takes a health-protective approach and assumes that lung and adrenal tumors arise independently, which allows for the use of MS Combo and avoids underestimating the risk for tumor formation. Medullary hyperplasia was not a consideration by OEHHA for deriving cancer potency factors.

ToxStrategies Comment 17:

3.7 OEHHA's use of the MS_Combo model is inappropriate due to differences in target-tissue dosimetry.

The combined modeling was based on OEHHA's conversion of inhaled doses to body burden (mg/kg-day). It seems highly unlikely that lung tumors, pancreatic tumors, and pheochromocytomas are the result of the same dose metric. Lung tumors are likely the result of direct site-of-contact effects, whereas pancreatic tumors may arise from either systemic effects or ingestion of cobalt metal. As mentioned above, it is conceivable that the pheochromocytomas are secondary to hypoxia-induced effects on oxygen

absorption in the lung. Therefore, combining risks based on body burden is unwarranted. As stated in Dr. Kenny Crump's analysis of MS_Combo (Versar. 2011. External peer review of EPA's MS-COMBO multi-tumor model and test report. Contract No. EP-C-7-025):

> USEPA generally prefers to utilize pharmacokinetic data on the dose to the target organ in its risk assessments. However, different tumor sites will have different internal doses and it will not be possible to take these differences into account properly with the current implementation of MS-COMBO. Conceptually, accounting for target organ doses would require incorporation of a quantitative physiologically-based pharmacokinetic (PBPK) model into the analysis… Consistent with the manner in which EPA normally uses PBPK data to convert from animals to humans, the animal tumor data would be modeled using tumor site-specific internal doses estimated from the animal PBPK model, and the BMD calculation would use the human PBPK model (implemented using the simple linear approximation) to calculate the human external BMD corresponding to these internal doses.

According to the USEPA RfC approach, lung tumors should be modeled as a pulmonary effect, whereas the pancreas is an extrarespiratory (i.e., systemic) tumor site. As noted above, the pheochromocytomas have questionable human relevance and may arise secondary to lung lesions. Without additional information, body burden might be a suitable dose metric for the pancreatic tumors and pheochromocytomas, but not for lung tumors. Unless each tumor response can be modeled in terms of its tissue-specific dosimetry, it makes little sense to model the tumors on a single exposure metric using MS_Combo.

In summary, OEHHA should not use MS_Combo to model pheochromocytomas with lung tumors; OEHHA should use dosimetric adjustments for particle deposition in the lung consistent with EPA guidance, to calculate and model HECs; and OEHHA should use a custom BMR in the observable range, rather than extrapolating over a 20¬fold dose range. Both EPA's BMD and OEHHA's cancer risk assessment guidance recognize the importance of selecting a BMR within or close to the observable range.

Response to ToxStrategies Comment 17:

A PBPK modeling analysis would also be preferred by OEHHA for extrapolation of tumor formation from rodent to humans, but a PBPK analysis has not been performed for either cobalt metal or cobalt sulfate heptahydrate. In addition, OEHHA must assume independence for lung and adrenal tumor formation, and assume systemic distribution

of inhaled cobalt to various organ systems where tumors have arisen (i.e., lung, adrenal medulla, pancreatic islets, leukemia). Thus, as explained in ***Response to ToxStrategies Comment #12***, OEHHA prefers to extrapolate from rodents to humans by converting the rodent CSFs to human equivalents using body weight ($BW^{3/4}$) scaling. MS Combo can then be used to assess multi-site tumor development and avoid underestimation of cancer risk.

OEHHA will revise the CSF for cobalt metal using the exact formula and a BMR of 15% lung, a BMR that provides a "viable" recommendation in BMDS version 3.1 (see ***Response to ToxStrategies Comment # 11 and 15***). This CPF derivation provides a more health protective cancer risk assessment than that suggested by ToxStrategies (i.e., and BMD_{78}).

Responses to Comments Received from the Cobalt Institute

DETAILED COMMENTS

Cobalt Institute Comment 1:

1 – In vivo genotoxicity of Co metal and Co compounds (referred to as "Co compounds" in the below comments)

The assumption of in vivo genotoxicity of Co compounds is based on data from studies with a low "Klimisch score", mainly based on non-relevant route of exposure (intra-peritoneal injection), low reliability based on flaws in reporting, and the fact that these studies did not follow OECD guidelines for genotoxicity testing. We would like to highlight to OEHHA an OECD review of 2014 (https://hpvchemicals.oecd.org/ui/handler.axd?id=e5e60085-1f3f-4df5-92f6-8f32c26c3082) which concludes lack of in vivo genotoxicity of Co compounds, following a stringent quality, reliability and relevance screening of the genotoxicity database of Co compounds. This conclusion is also reflected in recent publications [1, 2].

Response to Cobalt Institute Comment 1:

In vivo genotoxicity studies are a rather small subset of the overall genotoxicity study database for cobalt compounds, most of which are *in vitro* studies. *In vivo* genotoxicity studies summarized by OEHHA can be found in Section III and Table 6 of the Cobalt TSD.

OEHHA has already addressed the mixed results for *in vivo* genotoxicity studies by stating in the Cancer Hazard Evaluation, Section IV (page 44), "Recent rigorous *in vivo* studies (oral gavage and inhalation exposure) in cobalt-exposed rodents by Kirkland et al. (2015) and NTP (2014a) did not find evidence of chromosomal damage in bone marrow or erythrocytes, although *in vivo* chromosomal damage assays are regarded to be less sensitive than *in vitro* assays. The few genotoxicity tests conducted on blood lymphocytes of workers exposed to cobalt have been negative. Kirkland et al. (2015) suggest that protective processes that exist in whole animals compared to single cells are sufficient to prevent DNA damage resulting from ROS. Thus, other processes may be involved (e.g., inhibition of DNA repair) in the genotoxicity of cobalt. However, cells exposed to cobalt at the point of contact (i.e., pulmonary cells with inhalation exposure), as suggested by De Boeck et al. (2000), may be a better approach to investigate genotoxic damage caused *in vivo*."

The "Klimisch score" referred to by the Commenter was developed by Klimisch et al. (1997) of the chemical company BASF. The method assesses the reliability of toxicology-related studies by assigning scores of 1 to 4. Scores of 1 or 2 generaly indicate good laboratory practices (GLP) were used, while scores of 3 or 4 indicate poor GLP or insufficient methods description. A specific Klimisch score was not presented by Cobalt Institute (CI), but it can be presumed from Comment #1 that the *in vivo* genotoxicity studies that were positive for genotoxic effects had received a score of 3 or 4. OEHHA doesn't use these types of scoring systems, because they vary widely and haven't been generally vetted by OEHHA or US EPA. Also, scores that weigh GLP studies tend to favor industry studies over academic studies. Finally, Organisation for Economic Co-operation and Development (OECD) guidelines are used for European submissions of pesticide and pharmaceutical registration approval, and may only present the minimum data necessary for approval. OEHHA will consider all peer-reviewed studies, whether they adhere to OECD guidelines or not.

The embedded link in Comment #1 is a summary of cobalt toxicology data that appears to have been presented at a conference on October 3, 2014. The summary includes a short section that briefly notes method deficiencies of two published *in vivo* clastogenicity studies with soluble cobalt compounds (specific studies not identified). These deficiencies include biologically implausible time and dose-dependency of effects, non-physiological routes of exposure, and other deficiencies. More recent *in vivo* genotoxicity work that followed OECD guidelines were negative for genotoxicity.

Cobalt Institute Comment 2:

<u>Part 1 continued:</u>

Further work has very recently been conducted by the CI and Cobalt EU REACH Consortia (CoRC), using a novel assay specifically developed to distinguish between genotoxic versus non-genotoxic carcinogens. The assay is called "ToxTracker" and is a panel of mammalian stem cell lines (mouse embryonic stem cells) that contain different fluorescent reporters representing four distinct biological responses that are associated with carcinogenesis, i.e. general cellular stress, DNA damage, oxidative stress and the unfolded protein response [3]. The differential induction of the Green Fluorescent Protein (GFP) reporters as well as cytotoxicity of the tested compounds were determined by flow cytometry. Upregulation of hypoxia genetic markers was determined by quantitative Polymerase Chain Reaction (qPCR). Co metal powder and the highly soluble and bioavailable Co salt $CoCl_2$-hexahydrate were tested in this system. The results confirm the previous conclusions that Co compounds do not induce DNA damage, and instead are potent inducers of oxidative stress and hypoxia.

The ToxTracker data will be incorporated into an Adverse Outcome Pathway hypothesis for bioavailable Co compounds, and will be published before end of 2019. The ToxTracker method is currently undergoing OECD and ECVAM review and evaluation to become an OECD guideline method for testing of genotoxic versus non-genotoxic chemicals.

Response to Cobalt Institute Comment 2:

The suggested claim by CI is that cobalt carcinogenicity operates by a non-genotoxic pathway and would thus exhibit a threshold dose below which no tumors would be produced. OEHHA doesn't make a distinction between genotoxic and non-genotoxic carcinogens in the absence of data demonstrating that a non-genotoxic threshold mechanism is responsible for tumor production. OEHHA takes a health protective approach by using non-threshold models to extrapolate to low-dose human cancer risk from animal carcinogenicity data. It is uncertain what the mechanism(s) of mutagenicity is for cobalt, and whether it can (or should) be classified as a non-genotoxic carcinogen.

A study was recently published by Cappellini et al. (2018) and summarized in the OEHHA Cobalt TSD in which three cobalt-containing NPs were tested in the ToxTracker reporter assay to investigate mechanisms of genotoxicity. This ToxTracker assay also employed mouse embryonic stem cells, and contained six green fluorescent protein reporters specific for DNA damage, oxidative stress, protein damage, and cellular stress response. Cobalt metal NPs, and to a lesser extent cobalt(II) oxide NPs, caused an induction of the Srxn1-GFP reporter related to generation of ROS that can lead to DNA single strand breaks during the repair of oxidative DNA lesions. Cobalt metal and cobalt(II) oxide NPs also activated the Rtkn-GFP genotoxicity reporter that is associated with induction of DNA strand breaks.

However, the Bscl2-GFP reporter was not activated by the cobalt. Cappellini et al. (2018) reports that the induction of the Bscl2-reporter is associated with the ATR (ataxia telangiectasia and Rad3-related)/Checkpoint Kinase 1 (CHK1) DNA damage signaling pathway and identifies compounds that induce DNA replication blocking lesions. The absence of Bscl2-GFP reporter activation indicates that the Co NPs that were tested in this study did not directly bind to the DNA and interfere with DNA replication. However, the induction of the Rtkn-GFP reporter indicates a more severe oxidative stress response and resulting DNA damage compared to compounds that only induce the Srxn1-GFP reporter.

Overall, the authors concluded that the primary mechanism of genotoxicity by cobalt metal and cobalt(II) oxide NPs, but not cobalt(II,III) oxide, was induction of oxidative

stress that can lead to DNA strand breaks. Capellini et al. made no conclusion about genotoxic vs. non-genotoxic pathways of carcinogenicity for the cobalt NPs, but stated that further investigation regarding mutagenicity as a result of the DNA damage produced is warranted.

Cobalt Institute Comment 3:

2 - Assumption of "independence" of tumors in Co inhalation studies

There were exposure-concentration dependent increases in the incidences of benign and malignant pheochromocytoma (combined) in all substance-exposed male and female rats. This effect was not observed in mice. These tumors are well-established responses that are secondary to hypoxia and respiratory distress (adrenal pheochromocytoma in rats [4]).

In a statistical re-evaluation of nine, 2-year NTP inhalation studies, a range of lung effects (chronic active inflammation, interstitial fibrosis, alveolar epithelial hyperplasia, squamous metaplasia, proteinosis, and histiocytosis) and their association with pheochromocytoma was investigated. It was concluded that there is an overall association between lung impairment by any cause and an elevated incidence of adrenal pheochromocytoma in NTP inhalation studies. The elevated incidences of pheochromocytoma in rats after inhalation exposure to Co metal are considered to be rat-specific responses to respiratory distress, with no causal relationship to Co. Also, there is no indication for an involvement of genotoxic mechanisms in the induction of pheochromocytoma by chemicals in animals [4, 5].

Therefore, these tumors should not be assumed to be occurring independently, as this is not supported by the MoA leading to pheochromocytoma in inhalation studies and may lead to a severe overestimation of the potency of Co ion related carcinogenicity. The assumption of independence of the tumors warrants a closer look at all tumorigenic findings in the NTP inhalation studies with Co sulfate and Co metal powder:

Response to Cobalt Institute Comment 3:

This Comment is similar to one expressed by ToxStrategies (ToxStrategies Comment #14). NTP states in their Cobalt carcinogenicity study (NTP, 2016) that the development of pheochromocytomas in inhalation studies are not understood. In addition, NTP states in Behl et al. (2015) that, "Additional studies are needed to investigate whether the adrenal response is related to the presence of these extensive space occupying pulmonary lesions rather than due to a chemical specific response." Finally, the NTP Report on Carcinogens (2016) concluded, "… there is not enough

evidence to differentiate between a direct or indirect cause of adrenal gland neoplasms from cobalt exposure." Due to the lack of confidence for lung injury dependence of the rat pheochromocytomas, OEHHA has chosen a health protective approach by assuming that pheochromocytomas arise independently from the lung cancer and noncancer effects.

OEHHA searched NTP technical reports and found 11 additional NTP carcinogenicity studies that showed "some", "clear" or "positive" evidence of pheochromocytomas resulting from a chemical in feed or administered by gavage in which no pulmonary effects were found. In addition, an inhalation carcinogenicity study of Stoddard Solvent produced some evidence of pheochromocytomas in male rats, but no evidence of lung tumors or lung injury. Therefore, OEHHA cannot ignore the possibility that inhaled cobalt metal and cobalt compounds that are absorbed systemically and reach the adrenal glands could be a direct cause of pheochromocytoma.

The table below was derived from NTP carcinogenicity data for cobalt sulfate heptahydrate presented in Table 15 of the OEHHA Cobalt TSD. Note that OEHHA did not derive a draft CSF for cobalt metal using pheochromocytoma incidence data; these adrenal tumors are only used for deriving the CSF for cobalt sulfate heptahydrate. In female rats exposed to cobalt sulfate heptahydrate, the lung tumor CSF is considerably greater than the adrenal medulla tumor CSF. The CSF calculated for multisite lung/adrenal tumors is proposed by OEHHA to represent cancer risk for all soluble cobalt compounds. The risk is only modestly increased by combining the adrenal tumor data with the lung tumor data, and does not result in a "severe overestimation" as suggested by CI in their Comment.

"BMD_{05}, $BMDL_{05}$, rodent CSFs, and human CSFs for single-site and multi-site tumors in female rats resulting from 2-year inhalation exposure to cobalt sulfate heptahydrate

Tumor type	AIC[a]	*p*-value	BMD_{05} (mg/kg-day)[a]	$BMDL_{05}$ (mg/kg-day)	CSF - Rodent $(mg/kg\text{-}day)^{-1}$	CSF - Human $(mg/kg\text{-}day)^{-1}$
Rats						
Alveolar/bronchiolar						
Females	80.53	0.57	0.02456	0.01717	2.91	**11.75**
Adrenal medulla						
Females	100.07	0.60	0.1295	0.07852	0.64	**2.58**
Multisite: lung/adrenal tumors combined						
Females	NA	NA	0.02064	0.01504	3.32	**13.41**

Cobalt Institute Comment 4:

Part 2 continued:

Rare systemic tumors in the context of historical control data

Historical control data are needed to decide whether a tumor is “rare” (background rate of < 1%) or “common” (background rate > 1%) and are needed to interpret the significance especially of rare tumors and of marginally increased tumor incidences. In the NTP Co metal inhalation study, the tumors in kidney and pancreas can probably be considered “rare”, however, in this context, it needs to be outlined that there are no historical control data for the F344 NTac strain (the F344N colony at Taconic laboratories) and inhalation exposure route (in that strain) at NTP. In total, only two carcinogenicity studies were carried out at NTP with the F344 NTac rats, one by inhalation (the Co metal study) and one by p.o. route of exposure (TR 583, Bromodichloroacetic Acid, drinking water study). The “historical control” used by the NTP in the Co metal report consisted of only 100 animals, which actually includes the concurrent control (50 animals), with the addition of another 50 animals of study TR 583, exposed by a different route of exposure. This is not what would constitute a “historical control”. For comparison, a typical historical control database would consist of around 50 studies by the same route of exposure, and several thousand animals [6].

Response to Cobalt Institute Comment 4:

NTP regarded the kidney tumors in male rats to be equivocal evidence of carcinogenicity, and there was no evidence of kidney tumors in female rats. Because of the lack of clear findings for carcinogenicity, OEHHA did not derive a cancer potency factor for kidney tumors.

For pancreatic islet tumors, NTP found positive evidence for carcinogenicty in male rats, but only equivocal evidence for carcinogenicity in female rats. Thus, OEHHA derived a cancer potency factor for pancreatic islet tumors in male rats. Not only was there a positive trend for this tumor type in male rats, but there was also a statistically significant increase in adenoma, and adenoma and carcinoma (combined) at the two highest dose levels compared to controls: 2/50, 2/50, 10/48, 9/49 for 0, 1.25, 2.5, and 5 mg/m^3 groups, respectively, for adenoma and carcinoma (combined) incidence. The historical control incidence was 2/100 for both adenoma and adenoma and carcinoma (combined). NTP did not indicate anywhere in their report that the incidence data or historical control data for these tumors were deficient. Thus, OEHHA included the pancreatic islet tumor data in male rats for cancer risk evaluation.

Cobalt Institute Comment 5:

Part 2 continued:

Why are there no historical control data for the rat colony F344NTac used in the Co metal inhalation study?

Only one inhalation carcinogenicity study was ever conducted at the NTP with the F344NTac rat. It is important to realize that the F344NTac rats had developed a number of problems specific to this colony, including “declining fertility, sporadic seizure activity, and chylothorax” [7].

A specialty group set-up by the NTP (“rat breakout group”) notes that these issues “have occurred within the past 5 years in the NTP F344/N rat colony.” The NTP Co metal inhalation study range finders were finalized in 2005, meaning that the study design for the chronic study, including selection of rat strain and colony were already decided and underway by the time this report was issued. The report continues that “These issues are unique to our F344/N colony maintained at Taconic Farms, Inc. and to the best of our knowledge do not appear in other colonies maintained for commercial purposes at Taconic or other suppliers. The reasons for the development of these conditions in this specific colony have not been identified”. This led to the strong recommendation of the expert group to discontinue the use of this rat strain and colony, which was implemented by the NTP immediately.

Due to the increasing morbidity of the F344/NTac colony and the lack of historical control data, the occurrence of the systemic tumors in the Co metal study cannot be conclusively interpreted.

Response to Cobalt Institute Comment 5:

NTP did not express any concern that the strain of rat used in the cobalt metal study would affect the carcinogenicity incidence. Declining fertility, sporadic seizure activity, and chylothorax (a type of pleural effusion that results from lymph formed in the digestive system and accumulating in the pleural cavity) may affect non-cancer and reproductive findings but apparently did not have any bearing on the carcinogenicity.

However, OEHHA ultimately derived a cancer potency factor for cobalt metal based on the lung tumor incidence in male mice, because this was the most sensitive species and sex in the cobalt metal study. Thus, the concern expressed about the rat strain used in the cobalt metal carcinogenicity study is not particularly relevant for the CSF derived by OEHHA.

Cobalt Institute Comment 6:

Part 2 continued:

Common systemic tumors: Mononuclear cell leukemia (MNCL)

While there was an increase in MNCL at all exposure levels in female rats, the increase was not exposure level-related (incidence was highest at the lowest exposure level). In addition, there was no significant increase of MNCL in male rats. This finding did not occur in mice.

MNCL occurs with a high spontaneous background rate, and occurred at 42% and 36% in the controls, males and females, respectively. The incidence of MNCL is high across all exposure groups in the male rats, including controls (42%, 50%, 44%, 44% in control, 1.25, 2.5 and 5 mg Co/m^3 exposure groups, respectively); it is also high in all female rats with 36%, 62%, 61%, 59% in control, 1.25, 2.5 and 5 mg Co/m^3 exposure groups, respectively. The female control animals display an in fact somewhat low incidence of MNCL. These data reflect the general observation that MNCL is a common tumor type, and that Fisher rats are generally prone to developing MNCL as they age [8]. Extremely elevated incidences of MNCL have been previously observed in a number of chronic bioassays and 2-year carcinogenicity studies in F344 rats [9, 10]. The analysis of the spontaneous neoplasm incidences in F344 rats from chamber controls of 18 two-year inhalation studies carried out by the NTP revealed a frequent occurrence of MNCL in males (57.5%, range 34-70%) and in females (37.3%, range 24-54%) [9]. The data show that MNCL occurs in untreated aged rats at extremely high and variable rates. The conclusion that MNCL is a Co related tumor based on the data in female rats cannot be substantiated when taking into account the data from both sexes, and when taking into account the high and variable occurrence of this common tumor.

MNCL is uncommon in most other rat strains, and its background incidence in the Fisher rat has increased significantly over time. MNCL has not been found in other mammalian species and no histologically comparable tumor is found in humans [10]. In the light of the well-known occurrence of MNCL in the Fisher rat, this result does not suggest that this is an independently occurring tumor directly related to Co exposure.

Response to Cobalt Institute Comment 6:

NTP (2014) observed positive evidence for MNCL in female rats as a result of cobalt metal exposure. The incidences of MNCL were significantly increased in all exposed groups and exceeded the historical control incidence (35/100) for all routes of administration. It was noted that no clear exposure-concentration relationship was

seen. However, OEHHA observed a statistically significant positive trend ($p<0.05$) using the Cochran-Armitage trend test. NTP concluded that, “Although mononuclear cell leukemia is a common spontaneous neoplasm in F344 rats, the increased incidences in females in the current study were considered related to cobalt exposure”.

The fact that MNCL was not found in male rats or in mice is irrelevant, as sex and species tumor differences are often observed in carcinogenicity studies.

Contrary to what CI suggests in their Comment, a U.S. EPA report (2012) has noted that several authors have concluded that rat MNCL is similar to human natural killer cell (NK) LGL leukemia (Stromberg et al., 1985; Ishmael and Dugard, 2006; Thomas et al., 2007). So there does appear to be a human counterpart to rat MNCL leukemia.

Nevertheless, as noted in the above **Response to Comment #5**, OEHHA did not use the rat tumor incidence data (including the MNCL data) to derive a cancer potency factor, ultimately using the increased lung tumor incidence in male mice to derive a cancer potency factor for cobalt metal.

Cobalt Institute Comment 7:

Part 2 continued:

Kidney, adenoma/carcinoma combined

There was a minimal increase in the incidence of these tumors in male rats, although not statistically significant. Because of this slight increase an extended review using “step-sections” was conducted. Using these extended data there is no evidence of a carcinogenic response in male rats, which is supported by the lack of an increase in tubular hyperplastic changes or in kidney tumors in female rats or in male and female mice.

The neoplasms in the kidney were slightly above the concurrent control data, but not statistically significant and no overall positive trend was established. In the light of these arguments, these findings do not appear to warrant an assumption that these tumors are independently occurring and related to Co exposure.

Response to Cobalt Institute Comment 7:

OEHHA (and NTP) came to the same conclusion as CI regarding the kidney tumor results in male rats exposed to cobalt metal. Thus, OEHHA did not consider the kidney tumor data for cancer potency factor derivation.

Cobalt Institute Comment 8:

Part 2 continued:

Pancreatic islets

There was a small increase in islet-cell tumors in the mid- and high-dose male rats but not in female rats (a small but not statistically non-significant increase was seen in the highest dose group). Mice did not display this effect.

These tumors are rare, and they were seen for the first time in an NTP study. Also, the F344 NTac rat was used for the first, and only, time in an NTP inhalation study. It is impossible to interpret these findings, and the statement in the NTP report that there was “equivocal evidence of carcinogenic activity” is considered justified. This level of evidence should not be taken as a basis for a conclusion that these are independently occurring tumors caused by exposure to Co.

Apart from the pheochromocytoma, systemic tumors were observed exclusively in the inhalation study with Co metal powder. This may be related to the very high exposure concentrations (adjusted for Co equivalent, the lowest dose in the Co powder study was higher than the highest dose in the Co sulfate study), or it may reflect the health issues that have led to the immediate discontinuation of the use of the F344NTac colony in NTP cancer bioassays.

In summary, several aspects cast doubt on the interpretation that the individual systemic tumors are independent and directly related to Co:

- The predominant finding (adrenal pheochromocytoma) is a well-known response to respiratory distress and hypoxia
- For the remaining systemic tumors, the following points can be made:

  o There is a lack of an exposure-response relationship

  o They occurred only in one sex (either males or females) of the rats

  o There is a complete lack of a historical control database for this rat colony (F344NTac), making it impossible to conclude whether the systemic tumors are biologically relevant or statistically significant

  o This rat colony is uniquely sensitive and had developed a number of spontaneous diseases that immediately (after one inhalation study) led to the discontinuation of the use of this colony at NTP

Response to Cobalt Institute Comment 8:

NTP concluded that the increased pancreatic islet tumor incidence in male rats was related to cobalt metal exposure. Only the increased pancreatic islet tumor incidence in female rats was concluded to be equivocal evidence of carcinogenicity, and thus, were not used by OEHHA for cancer potency factor derivation. The lack of pancreatic islet tumors in exposed mice is irrelevant, as sex and species differences are often observed for tumor types in carcinogenicity studies.

Some systemic tumors observed in rats were considered by NTP (and OEHHA) to be related to cobalt metal exposure. These include pheochromocytoma in male and female rats, pancreatic islet tumors in male rats, and MNCL in female rats. As stated in Response to Cobalt Institute Comment #3, there is not enough evidence to differentiate between a direct or indirect cause of adrenal gland neoplasms from cobalt exposure. Thus, OEHHA takes a health protective approach as assumes the adrenal tumors arise independently from the lung cancer and noncancer effects.

As noted above, OEHHA ultimately did not use the rat cancer data to derive a cancer potency factor for cobalt metal, instead relying on the most sensitive species and sex (i.e., lung tumors in male mice).

Cobalt Institute Comment 9:

<u>3 – Assumption of low solubility of Co metal powder</u>

While Co metal powder is poorly soluble in water, it is in fact moderately to highly soluble in biological fluids, such as interstitial, alveolar or lysosomal artificial lung fluids. Data on the bioelution of several Co compounds in lung fluid has led to the grouping of Co metal powder with the “soluble salts” (Co sulfate, Co chloride, Co nitrate and Co acetate) in one group of Co compounds classified as inhalation carcinogens (Carc 1B). This group of compounds is characterized by the induction of an inflammatory response and hypoxia in the lung following inhalation exposure. The similarity in effects caused by this group of substances has led to the conclusion that the toxicity of Co compounds is related to the Co ion, and that the magnitude of effect is related to the Co ion dose-to-target. This also inherently assumes that dose-to-target is critical for the magnitude of effect, and not differences in the potency between Co substances. This assumption is confirmed by the evaluation of the dose-response of Co exposure (from Co sulfate and Co metal powder) across all exposure concentrations in both NTP studies. The combination of both Co compounds into one dose response curve results in very good model fit, and the indication that the model is able to predict exposure-responses at

relevant (low) exposures. A detailed report on benchmark dose (BMD) modeling of the complete animal dataset (Co metal powder and Co sulfate) is appended to these comments.

It is important to note that there are substances with negligible solubility in biological fluids (e.g., Co_3O_4 and CoS). Bioelution data exist indicating that these "biologically insoluble" substances should not be grouped with Co metal powder for the endpoint inhalation toxicity. These bioelution data are currently being written up into a manuscript for publication (together with the mechanistic data generated by the ToxTracker assay mentioned earlier). CI is willing to share / discuss bioelution, but not to put data in the public domain before publication.

Response to Cobalt Institute Comment 9:

Once cobalt is inhaled, how it is absorbed and distributed in the airways and airway epithelial cells depends on whether it is a water-soluble cobalt compound, or an insoluble cobalt compound. It is postulated that this difference in absorption and distribution between the two forms of cobalt is an important factor in its toxicity and carcinogenicity. The reasoning for categorizing cobalt metal with insoluble cobalt compounds (e.g., cobalt oxides) rather than soluble cobalt compounds is as follows:

On page 2 of the Cobalt cancer IUR factor document, OEHHA writes, "Water-soluble cobalt compounds reaching the alveoli following inhalation will dissolve in the alveolar lining fluid and release the cobalt ion (Kreyling et al., 1986; Stopford et al., 2003). Water-insoluble cobalt compounds (e.g., cobalt oxides) and cobalt metal reaching distal airways and alveoli may dissolve intracellularly in the acidic environment of lysosomes (pH 4.5 to 5) following uptake via endocytosis by macrophages and other epithelial cells (Kreyling et al., 1990; Ortega et al., 2014)." In the OEHHA Cobalt TSD, cobalt compounds that have a water solubility of >100 mg/L at 20˚C are considered water-soluble. Insoluble/poorly soluble cobalt compounds are defined as having a water solubility of ≤100 mg/L.

As presented by NTP (2016), physical and chemical properties of cobalt metal and cobalt compounds can be described by their water solubility and bioaccessibility in lysosomal fluid (Table 1). OEHHA proposes using this simple method of categorization to to assign a CSF to a cobalt compound.

Table 1. Solubilities of some cobalt compounds (NTP, 2016)

Molecular Formula	Form of Cobalt (Metal or Cobalt Compound)	Water solubility (g/100 cc)	Solubility in lysosomal fluid
Co	Cobalt metal particles/dust	0.00029	100
CoO	Oxide (II)	0.00049	92.4
Co_3O_4	Oxide (II,III)	0.00016	2-50%
$CoSO_4$	Sulfate (heptahydrate)	60.4	100
$CoCl_2$	Chloride (hexahydrate)	45	100
$Co(C_2H_2O_2)_2$	Acetate (tetrahydrate)	34.8	80
CoN_2O_6	Nitrate (hexahydrate)	67.0	100

Bioaccessibility information of cobalt compounds in interstitial and alveolar fluid is also helpful but this type of data is not nearly as common as water solubility data, and is quite limited for some cobalt compounds. Stopford et al. (2003) reported alveolar and interstitial fluid bioaccessibility of 4.8 and 4 percent, respectively, for extra fine cobalt metal (particle size 7.20 µm). For comparison to the above table, this was calculated by OEHHA to be roughly 0.096 g/100 cc and 0.08 g/100 cc bioaccessibility for alveolar and interstitial fluid, respectively. These data suggest greater solubility of cobalt metal in alveolar and interstitial fluids compared to distilled water, although differences in particle size and surface area could be a factor. However, how the lung handles inhaled cobalt metal is the main factor in determining carcinogenicity. Similar to water-insoluble cobalt(II) oxide, several *in vitro* studies show that cobalt metal particles are mainly internalized in lung cells by endocytosis (Cappellini et al. 2018; Colonago et al., 2008; Sabbioni et al., 2014; Ortega et al. 2014).

For cobalt nanoparticles (and microparticles), a "Trojan-horse"-type mechanism has been proposed in which the particles *in vitro* interact with proteins on the surface of cells and readily taken up (Ponti et al., 2009; Colognato et al., 2008; Ortega et al. 2014). This resulted in a 50- to 140-fold greater cellular uptake and intracellular release of cobalt ion from insoluble cobalt (i.e., cobalt(II) oxide) vs. uptake of extracellular ions from a soluble cobalt compound (cobalt chloride)." Co ions from soluble cobalt compounds are actively uptaken into cells only after saturation of binding sites of molecules (e.g., albumiin, histidine) in the extracellular milieu (Sabbioni et al., 2014b). We go on to state on Page 18 of the Cobalt TSD that, "Further research suggests internalized cobalt metal nano- and micro-particles diffuse to subcellular organelles and release cobalt ion in millimolar concentrations in nuclei and mitochondria (Sabbioni et al., 2014a,b)." On page 28 of the Cobalt TSD we summarize that, "…in vitro genotoxicity studies by Smith et al., (2014) led to the conclusion that solubility appears to play a role in cobalt-induced lung cell genotoxicity and suggests soluble and insoluble forms of cobalt may have different carcinogenicity potentials."

Regarding the comment of low solubility cobalt compounds, NTP (2016) noted that very low bioaccessibilities of <2% have been reported cobalt(II, III) oxide (Co_3O_4) and some other cobalt compounds. However, NTP (2016) was reporting unpublished information from the Cobalt Development Institute and it was unclear what physiological fluid was employed to estimate the bioaccessibility. The NTP (2016) goes on the state that, "However, other, more informative tests with more physiologically relevant test conditions (e.g., two-week studies with 0.3 µm particles in culture medium in the presence of alveolar macrophages) have reported 50% solubility for cobalt(II, III) oxide." In this study by Kreyling et al. (1990), roughly half the cobalt particles ingested by the macrophages in culture had become solubilized over a two week period. In an *in vitro* study with BEAS-2B human lung cells, Ortega et al. (2014) found that cobalt(II, III) oxide particles were partially solubilized at low pH within lysosomes, leading to cobalt ion release. Solubilized cobalt was detected within the cytoplasm and the nucleus. The intracellular solubilized cobalt content was small compared with the intracellular particulate cobalt content. However, the authors were able to demonstrate that this minute fraction of intracellular solubilized cobalt lead to cytotoxicity. Thus, OEHHA categorizes cobalt(II,III) oxide as an insoluble carcinogenic cobalt compound and assigns to it the cancer potency factor derived for cobalt metal.

Cobalt Institute Comment 10:

<u>4 - Calculation of BMDL5 with Co metal data only</u>

A serious concern arises related to the use of the BMD model in the context of the Co metal data alone. Doses/exposures are needed that produce different effect sizes providing information on both the lower and higher part of the dose–response relationship to characterize the full dose–response relationship [11]. Limitations in data can arise from a relatively high response at the lowest dose [11], and it can be concluded that using more but smaller dose groups definitely does not deteriorate BMD precision, but rather may have a positive impact on the performance of the study [12]. Indeed, it has been suggested that the magnitude of uncertainty of the BMD estimate, as indicated by the BMDL–BMDU ratio, should be used as a tool for evaluating the statistical quality of the underlying data [13], and the utility of a BMDL as a reference PoD for regulatory decision-making [13-15].

In the Co metal powder study, at the lowest dose, 30% of the female rats and 50% of the male rats had lung tumors. Extrapolation from high dose/high response data into areas of lower responses (e.g. BMD10 or 05) that are this far outside the data results in high uncertainty and very large differences between the BMDL-BMDU ratio (BMD upper and lower confidence limits).

A BMDL05 calculation based on Co metal data (male rats) alone shows that the ratio between BMDL and BMDU at 5% risk is 24, demonstrating the high uncertainty of the modeled BMD05 values. This uncertainty is significantly reduced, with a BMDL-BMDU ratio of 3.75, when the Co sulfate data are included in the dose response modeling. The reduction in the uncertainty is a result of the Co sulfate exposures, which were all lower than those applied in the Co metal study when compared on the Co equivalent basis. The BMD5 modeling using all data (Co sulfate and Co metal powder), both rats and mice, males and females, reduces the BMDL-BMDU ratio to 3. There appears to be a good dose-response fit across all studies (Co metal powder and Co sulfate, rats-mice, male-female), rather than an elevated potency of Co metal powder versus Co sulfate. This indicates that the responses are related to the Co equivalent exposure concentration, and not to a difference in potency between Co metal powder and Co sulfate.

Response to Cobalt Institute Comment 10:

Regarding the use of a BMDL-Benchmark Dose Upper Confidence Limit (BMDU) ratio to assess the uncertainty in a benchmark dose response, such as a BMDL05, OEHHA does not disagree that this type of assessment is useful. However, in the US EPA benchmark dose software, the results are flagged with warnings if the BMD is 3x lower than lowest non-zero dose and BMDLs are 10x lower than lowest non-zero dose. OEHHA is using this US EPA guidance in their BMD software to determine acceptable model fits to the data.

The Cobalt Institute presents benchmark dose modeling of the male rat lung tumor incidence data at the end of their Comments Section. Although the background incidence of lung tumors in male mice was greater than in male rats, OEHHA found that the lung tumor incidence in male mice resulted in a higher cancer slope factor (CPF) following adjustment to the human equivalent concentration (HEC). OEHHA will use the male mice results to establish a CPF for cobalt metal and particulate cobalt compounds. OEHHA recognizes that a BMR of 5% for male mice lung tumors is flagged as "questionable" in the benchmark software, due to a BMD that is 3x lower than lowest non-zero dose and a BMDL that is 10x lower than lowest non-zero dose. As described in the ***Response to ToxStrategies Comment #15*** below, OEHHA uses a BMR of 15% with the exact formula for the calculation of the cancer slope factor: β1: -ln(1 BMR)/BMDL. This formula accounts for the increased curvature in the dose-response relationship at higher doses and BMRs. However, use of the exact formula for the animal cancer slope factor (CSF_a), shows that the choice of BMR (5%, 10% and 15% response) had no effect on the value of the cancer slope factor to calculate the CSF.

CI combines both the cobalt metal and cobalt sulfate heptahydrate lung tumor incidence data in male rats to derive a single cobalt BMDL value of 0.12 mg/kg-day. The BMR chosen was 5%, with a 90% confidence interval around the BMD ($BMDL_{10}$). Typically, OEHHA would have chosen a 95% confidence interval around the BMD. Although not calculated by CI, this BMDL would result in a rodent CSF of 0.42 $(mg/kg\text{-}day)^{-1}$ (0.05 / 0.12). For comparison, based on the methods described in the draft OEHHA Cobalt TSD, OEHHA derived rodent CSFs of 4.57 and 0.74 $(mg/kg\text{-}day)^{-1}$ for cobalt metal and cobalt sulfate heptahydrate (normalized to content of cobalt), respectively.

As outlined in ***Response to ToxStrategies Comment #9*** above, the lung tumor incidence slopes for cobalt metal appear steeper than the lung tumor incidence slopes for cobalt sulfate heptahydrate for both rats and mice (see Figure 2). This would suggest that cobalt metal is a more potent carcinogen than cobalt sulfate heptahydrate. This finding is supported by the *in vitro* genotoxicity data, which suggests a different mechanism, or modes of entry into cells, for the two cobalt forms, leading researchers to conclude that cobalt metal would be a more potent carcinogen compared to soluble cobalt compounds such as cobalt sulfate heptahydrate (Ponti et al., 2009; Colognato et al., 2008; Ortega et al. 2014; Smith et al.2014; Sabbioni et al., 2014b). Thus, OEHHA derived IURs separately for cobalt metal and cobalt sulfate heptahydrate.

Responses to Comments Received from the Color Pigments Manufacturers Association (CPMA)

CPMA Comment 1:

CPMA strongly supports the comments of the Cobalt Institute on the Draft Document. As proposed, the Draft Document uses multiple layers of excessively conservative assumptions which would grossly overestimate the risks for many Cobalt compounds and products, including complex inorganic color pigments containing Cobalt. As discussed by the Cobalt Institute, the Draft Document sets unrealistically conservative parameters for mutagenicity, solubility and independence of tumors, which, when taken together, generate a disproportionate outcome which is not relevant to any reasonable estimation of risk.

Assessments such as the Draft Document can have unanticipated negative impacts on the environment and the economy. Overly conservative regulation can act to force inappropriate substitutions which unintentionally bring more hazardous and unevaluated chemistries to the market.

Response to CPMA Comment 1:

OEHHA does not agree that the CSF methodology used would "grossly overestimate" the cancer risk and generate a "disproportionate outcome". The Cobalt Institute (CI) combined the NTP cobalt metal and cobalt sulfate heptahydrate cancer data for lung tumors in male rats to derive a single CSF for presumably all cobalt compounds that would be soluble in physiological fluids. A rodent CSF of 0.42 $(mg/kg\text{-}day)^{-1}$ is calculated by CI by this method (See Response to Cobalt Institute Comment #10). OEHHA calculated rodent CSFs of 4.57 and 0.74 for cobalt metal and cobalt sulfate heptahydrate, respectively. The CI rodent CSF and the OEHHA cobalt sulfate heptahydrate CSF are not that far apart.

As depicted in Figure 2 in ToxStrategies Comment #9, the rat and mouse cobalt metal cancer incidence slopes appear steeper than the rat and mouse cobalt sulfate heptahydrate cancer incidence slopes. This finding indicates cobalt metal is a more potent carcinogen than cobalt sulfate heptahydrate. The *in vitro* genotoxicity data supports this finding. As noted in Response to Comment #9, differences in cellular uptake between soluble and insoluble forms of cobalt have been proposed as a reason for differences in cancer potency. It has been shown that cobalt nanoparticles *in vitro* interact with proteins on the surface of cells and are readily taken up by those cells (Ponti et al., 2009; Colognato et al., 2008). This resulted in a 50- to 140-fold greater

cellular uptake and intracellular release of cobalt ion from insoluble cobalt (i.e., cobalt(II) oxide) vs. uptake of extracellular ions from a soluble cobalt compound (cobalt chloride). Further research suggests internalized cobalt metal nano- and micro-particles diffuse to subcellular organelles and release cobalt ion in millimolar concentrations in nuclei and mitochondria (Sabbioni et al., 2014a,b)." Smith et al., (2014) suggested that solubility appears to play a role in cobalt-induced lung cell genotoxicity, and that soluble and insoluble forms of cobalt may have different carcinogenicity potentials. Thus, OEHHA believes that CSFs should be calculated separately for cobalt metal and cobalt sulfate heptahydrate.

OEHHA uses the best data available to estimate the cancer risk of chemicals, regardless of the possible ramifications. Once a CSF has been determined for a chemical, regulatory agencies make decisions on how to manage the potential health risks.

CPMA Comment 2:

In particular, CPMA agrees with and specifically supports the Cobalt Institute comments on the unsubstantiated Draft Document conclusion that Cobalt and Cobalt compounds are genotoxic, based on studies using non-OECD guidelines such as the comet assay. CPMA agrees with the Cobalt Institute that Cobalt is not mutagenic and has not been shown to exhibit in vivo genotoxicity in OECD guideline studies. The mode of action which has linked certain Cobalt exposures with cancer in animals is through inflammation of the exposed tissues. The assumption that Cobalt is genotoxic vastly overstates the risk posed by Cobalt.

Response to CPMA Comment 2:

As presented in the OEHHA cobalt TSD, there are many *in vitro* studies that demonstrated the genotoxicity of cobalt compounds. OEHHA summarizes both OECD and non-OECD guideline studies. We do not specifically exclude non-OECD guideline studies. Both CPMA and CI appear to place a significant amount of weight on the *in vitro* and *in vivo* studies by Kirkland et al. (2015). Kirkland et al. (2015) used some OECD guidelines to examine the genotoxicity and mutagenicity of a number of cobalt compounds and cobalt metal. These authors found that cobalt sulfate heptahydrate and cobalt octoate produced oxidative DNA damage in human A549 cells. DNA damage was determined using the human 8-hydroxyguanine DNA-Glycosylate 1 (hOGG1) modified comet assay, although it was unclear from the report if the method used was based on OECD guidelines. The same authors employed OECD guidelines to observe chromosomal damage in human lymphocytes *in vitro* following exposure to cobalt acetyl

acetonate, and with some qualifications, cobalt resinate and cobalt oxyhydroxide as well. Thus, cobalt compounds are found to be genotoxic by researchers that use OECD or non-OECD guidelines.

Kirkland et al. (2015) also examined the potential for mutagenicity of cobalt compounds using bacterial and mammalian cell gene mutation tests, although it was unclear from the report if OECD guidelines were specifically used. As summarized in the OEHHA cobalt TSD, Kirkland et al (2015) found all cobalt compounds examined were negative for mutagenicity. This is not surprising, given that some previous mutagenicity tests of cobalt compounds by other researchers were also negative, or got only weakly positive results.

NTP (1998) found that cobalt sulfate heptahydrate was mutagenic in S. typhimurium TA100 with and without S9, but was not mutagenic in TA98 or TA1535 strains with or without S9. NTP (2014) also investigated the mutagenicity of cobalt metal. Without S9, cobalt produced an equivocal response with S. typhimurium TA100, but was weakly mutagenic with the TA98 strain. With S9, no mutagenic activity was observed in either S. typhimurium strain. Hong et al. (2015) suggested the lack of mutagenicity in S. typhimurium with S9 could be related to radical scavenging enzymes (e.g., glutathione peroxidase) contained within the S9 mix and/or binding of cobalt to S9 proteins.

OEHHA addressed the *in vivo* mutagenicity studies in **Response to Cobalt Institute Comment #1** and in the OEHHA cobalt TSD where we note, "Recent rigorous *in vivo* studies (oral gavage and inhalation exposure) in cobalt-exposed rodents by Kirkland et al. (2015) and NTP (2014) did not find evidence of chromosomal damage in bone marrow or erythrocytes, although *in vivo* chromosomal damage assays are regarded to be less sensitive than *in vitro* assays. The few genotoxicity tests conducted on blood lymphocytes of workers exposed to cobalt have been negative. Kirkland et al. (2015) suggest that protective processes that exist in whole animals compared to single cells are sufficient to prevent DNA damage resulting from ROS. Thus, other processes may be involved (e.g., inhibition of DNA repair) in the genotoxicity of cobalt. However, cells exposed to cobalt at the point of contact (i.e., pulmonary cells with inhalation exposure), as suggested by De Boeck et al. (2000), may be a better approach to investigate genotoxic damage caused *in vivo*."

CPMA Comment 3:

The Draft Document adopts the position that the "Cobalt ion following inhalation is considered to be the primary factor for cancer risk (NTP, 2016)". The Draft Document applies inhalation factors to all water soluble compounds, with a solubility greater than

100 mg/L, and to all water insoluble compounds, with water insolubility less that 100 mg/L.

CPMA believes that it is inappropriate for OEHHA to categorize all compounds with solubilities lower than 100 mg/L as essentially the same for inhalation risk assessment. This one-size-fits-all approach to regulation overstates the risk for many compounds and products, such as complex inorganic color pigments which do not yield significant amounts of bioavailable Cobalt.[2]

[2]For example, see the study by D. Steinhoff and U. Mohr, entitled "On the Question of a Carcinogenic Action of Cobalt Containing Compounds", "Exp. Pathol.", Vol. 41, 169-174, 1991, which compared Cobalt Oxide and the pigment identified as Cobalt Aluminum Chrome Spinel in an intratracheal instillation study in rats.

Response to CPMA Comment 3:

OEHHA states on page 2 of the cobalt TSD, “Bioaccessibility of the cobalt ion following inhalation is considered to be the primary factor for cancer risk (NTP, 2016). Thus, any cobalt compound inhaled that releases the cobalt ion in pulmonary fluids presents an inhalation cancer risk.” Therefore, if a cobalt compound is not considered soluble in alveolar, interstitial or lysosomal fluids, it is unlikely to present a cancer risk as a result of release of the cobalt ion.

Cobalt aluminum chrome spinel is made by calcining at 2400°F a mixture of cobalt(II) oxide, chromium(III) oxide, and aluminum(III) oxide in varied ratios forming an interdiffused crystalline spinel matrix. The spinel described by Steinhoff and Mohr (1991) contained 24% cobalt. The solubility of cobalt aluminate spinel (CASRN 68186-86-7) was investigated by Stopford et al. (2003). This spinel contained 23.6% cobalt and appears to be a similar, or the same, compound as that examined by Steinhoff and Mohr (1991). It was found to be only 0.089% soluble in lysosomal and gastric fluids (pH 4.5), and even less so in alveolar and interstitial fluids. In addition, the unique crystalline structure of cobalt aluminum spinel suggests that its properties may not necessarily reflect the properties of the component metals or oxides. This physical/chemical change is a situation similar to cobalt alloys, where the properties of the component metals may not reflect the toxicity of cobalt metal alone.

IARC (2006) concluded there is inadequate evidence for the carcinogenicity of cobalt-aluminum chromium spinel. Studies reviewed by IARC included Steinhoff and Mohr (1991), where intratracheal instillation of this spinel in rats was associated with the occurrence of a few pulmonary squamous-cell carcinomas (3/100). No pulmonary tumors were observed in 100 untreated or 100 saline controls. Intraperitoneal injection

of cobalt-chromium-aluminum spinel in rats produced a few local malignant tumors. A study in workers exposed to cobalt aluminum spinel provided, at best, equivocal evidence for an increased risk of lung cancer associated with exposure to cobalt spinel (Tuchsen et al. 1996). Both of these studies are summarized in the OEHHA cobalt TSD.

Overall, in regard to its carcinogenicity, cobalt aluminum spinel appears to have properties similar to alloys and has very low solubility in lysosomal fluid (0.089%, Stopford et al., 2003). These spinels will not be included with the IURs derived for cobalt and cobalt compounds.

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