National Toxicology Program

Peer Review of Draft Report on Carcinogens (RoC) Monograph on Cobalt and Certain Cobalt Compounds

July 22, 2015

National Institute of Environmental Health Sciences
Research Triangle Park, NC

Peer-Review Report

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I. Attendees*

Peer Review Panel

Melissa A. McDiarmid (Chair), University of Maryland School of Medicine
Lisa DeRoo, University of Bergen, Norway
Robert F. Herrick, Harvard School of Public Health
C. William Jameson, CWJ Consulting, LLC
Clark Lantz, University of Arizona
John LaPres, Michigan State University
Marie-Elise Parent, Université du Québec
Michael V. Pino, Independent Consultant
John Pierce Wise, University of Louisville
Anatoly Zhitkovich, Brown University

National Toxicology Program Board of Scientific Counselors Liaison

George B. Corcoran, Wayne State University (by webcast)

Other Federal Agency Staff

Paul Howard, U.S. Food and Drug Administration
Tania Carréon-Valencia, National Institute for Occupational Safety and Health

Technical Advisors

Janet Carter, Occupational Safety and Health Administration (by telephone)

National Institute of Environmental Health Sciences Staff

Mamta Behl	Ramesh Kovi	Georgia Roberts	Vickie R. Walker
Abee Boyles	Kelly Lenox	Andrew Rooney	Porscha Walton
John Bucher	Ruth Lunn	Diane Spencer	Lori White
Natasha Catlin	Robin Mackar	Kyla Taylor	Mary Wolfe
Michael DeVito	David Malarkey	Kristina Thayer	
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Alton Peters, ILS
Pamela Schwingl, ILS

Public Attendees

Sarah Bradford, University of North Carolina, Chapel Hill
Ruth Danzeisen, Cobalt Development Institute
Sang-Tae Kim, Ashland, Inc.
Thomas Shaw, Sandvik Machining Solutions
Scott Sieber, Ashland, Inc.

*The meeting was webcast. Individuals who viewed the webcast are not listed, except as noted.

II. Welcome and Introductions

The National Toxicology Program (NTP) Peer-Review Panel ("the Panel") for the Draft Report on Carcinogens (RoC) Monograph on Cobalt and Certain Cobalt Compounds convened on July 22, 2015, in Rodbell Auditorium, Rall Building, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Melissa McDiarmid served as chair. Dr. George Corcoran attended by webcast as the NTP Board of Scientific Counselors (BSC) liaison. Dr. Tania Carréon-Valencia attended as liaison from the National Institute for Occupational Safety and Health (NIOSH). Dr. Paul Howard attended as liaison from the U.S. Food and Drug Administration (FDA). Representing NTP were Dr. John Bucher, NTP Associate Director; Dr. Mary Wolfe, Deputy Division Director for Policy; Dr. Kristina Thayer, Deputy Division Director for Analysis; and Dr. Ruth Lunn, Director, Office of the RoC. Dr. Lori White, Office of Liaison, Policy and Review, served as the Designated Federal Official.

Dr. McDiarmid called the meeting to order at 9:00 a.m., welcomed everyone, and asked all attendees to introduce themselves. Dr. Bucher welcomed and thanked the attendees for their service on the Panel. Dr. White read the conflict of interest policy statement and briefed the attendees on meeting logistics. Dr. McDiarmid briefed the Panel and the audience on the format for the peer review.

III. Process for Preparing the Draft RoC Monograph

Dr. Ruth Lunn, Division of the NTP (DNTP), presented background information about the RoC and on the process and methods used to prepare the Draft RoC Monograph on Cobalt and Certain Cobalt Compounds. She noted that the RoC is congressionally mandated and identifies substances that pose a cancer hazard for U.S. residents. It is prepared for the Secretary of Health and Human Services (HHS) by NTP and is cumulative, including the profiles for newly listed substances and for all substances listed in previous reports.

Dr. Lunn outlined the four-part formal process for preparing the RoC, which consists of the following steps: (1) nomination and selection of candidate substances, (2) scientific evaluation of the candidate substances, (3) public release and peer review of the draft RoC monographs, and (4) submission of the substance profiles to the HHS Secretary for review and approval. The process incorporates public comment, scientific input, and peer review of the scientific information.

Dr. Lunn noted that for every candidate substance proposed for review, a concept document is written that explains the rationale and proposed approach for the review. Once a substance is formally selected for review, a draft RoC monograph is prepared, which consists of two parts: (1) a cancer hazard evaluation component, which assesses the quality of the studies, reaches level-of-evidence conclusions, and proposes a preliminary listing recommendation, and (2) the draft substance profile, which summarizes the key studies on which the listing recommendation is based and provides information on exposure. If the substance is listed in the RoC, the profile becomes part of the RoC.

Dr. Lunn outlined the steps of the process that had been completed for selection and evaluation of cobalt and certain cobalt compounds. Nomination of cobalt as a candidate substance was

based on the NTP bioassay of the carcinogenicity of cobalt metal (final report published in 2014). The nomination was announced and public comment solicited in September 2013. A draft concept document was developed, in which the scope of the nomination was expanded to "cobalt," leaving open the question of which cobalt compounds would be included. The draft concept document was released for public comment in March 2014 and presented to the BSC in April 2014. Cobalt was selected for further review based on widespread exposure and an adequate database.

To aid in defining the candidate substance, public comment was solicited via a website, and an informational group of federal government scientists with expertise on cobalt was convened. The informational group recommended defining the candidate substance as "cobalt and certain cobalt compounds," where "certain" refers to those cobalt compounds that release cobalt ions *in vivo.* The informational group also agreed that cancer studies on cobalt alloys and radioactive cobalt should be excluded from the review because of potential confounding. This treatment of cobalt compounds as a class is based on mechanistic data on the effects of cobalt ions. Dr. Lunn noted that cobalt sulfate, currently listed in the RoC as *reasonably anticipated to be a human carcinogen,* is included in this evaluation.

Dr. Lunn reviewed the protocol for preparing the cancer hazard evaluation, focusing on the literature search strategy and the approach for assessing the data on exposure and carcinogenicity. She reviewed the RoC criteria for listing a substance in the RoC as *known to be a human carcinogen* or *reasonably anticipated to be a human carcinogen.*

The charge to the Peer-Review Panel was as follows:

- To comment on the draft cancer evaluation component, specifically, whether it is technically correct and clearly stated, whether the NTP has objectively presented and assessed the scientific evidence, and whether the scientific evidence is adequate for applying the listing criteria.
- To comment on the draft substance profile, specifically, whether the scientific evidence supports the NTP's preliminary RoC listing decision for the substance.

The Panel would be asked to vote on the following questions:

- Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from cancer studies in humans.
- Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from cancer studies in experimental animals.
- Whether the scientific evidence supports the NTP's preliminary policy decision on the RoC listing status.

Dr. Lunn noted that the draft monograph would be revised based on NTP's review of the peer-review comments. The revised monograph, the peer-review report, and the NTP's response to the peer-review report are provided to the BSC, after which the monograph is finalized.

IV. Public Comments

IV.A. Written Public Comments

NTP received a written public comment on the draft monograph from the Cobalt Development Institute (CDI) on July 9, 2015, which was posted to the meeting webpage and distributed to the Panel. The Inorganic Pigments Consortium submitted a written comment at the meeting, supporting the comment from CDI. The comment was distributed to the Panel and posted on the meeting webpage.

IV.B. Oral Public Comments

Dr. Ruth Danzeisen presented comments on behalf of CDI, a not-for-profit trade association representing cobalt producers, users, and recyclers, which conducts research on human health and environmental effects of cobalt. She noted that the European Union classifies cobalt sulfate as an inhalation carcinogen Category 1B (presumed carcinogenic potential to humans), based on the 1998 NTP bioassay of cobalt sulfate. Four other soluble inorganic cobalt salts also are classified as Category 1B inhalation carcinogens, based on the read-across approach. In addition, CDI classifies cobalt metal as a Category 1B inhalation carcinogen, based on the 2014 NTP bioassay of cobalt metal. The question is which other cobalt compounds should be included in this group. CDI has evidence that the tested substances are not representative of all commercially available cobalt compounds.

Dr. Danzeisen stated that new CDI-sponsored research on the mutagenicity and genotoxicity of cobalt compounds found no relevant *in vivo* genotoxicity, regardless of the compounds' solubility. She suggested that this research, reported in a paper by Kirkland *et al.* and recently accepted for publication in *Regulatory Toxicology and Pharmacology,* be considered in the revision of the draft monograph.

These results prompted CDI to propose a non-genotoxic mode of action for cobalt carcinogenicity, based on CDI data on acute responses to cobalt inhalation and on data from the NTP subacute, subchronic, and two-year exposure studies in rodents. Dr. Danzeisen stated there is overwhelming evidence for inflammation as the major response, with sustained cobalt exposure leading to chronic inflammation, reparative hyperplasia, metaplasia, and eventually cancer. This response shows both temporal and dose-response relationships with exposure. She stressed the importance of distinguishing between genotoxicity and inflammation as the carcinogenic mode of action, because this will be an important consideration in later risk and exposure assessments.

CDI has tested over 30 cobalt substances for solubility and ion-release behavior in a variety of biological fluids. There is evidence for three potential groups of cobalt substances with respect to lung toxicity: (1) water-soluble substances (e.g., cobalt sulfate), which dissolve in neutral fluids, (2) poorly water-soluble particles (e.g., cobalt metal powder), which dissolve in acidic (e.g., lysosomal) fluids, and (3) insoluble substances (e.g., spinel-type oxides, pigments, and, arguably, vitamin B_{12}), which do not release cobalt ions in either neutral or acidic fluids. Evidence for the existence of this third group comes from both *in vitro* and *in vivo* data. Dr. Danzeisen noted that the two substances tested by NTP for carcinogenicity are relatively

high releasers of cobalt ions in lysosomal fluid, with release by the poorly water-soluble cobalt metal exceeding that of the water-soluble cobalt sulfate, and are not representative of cobalt substances as a whole.

Dr. Danzeisen said that although NTP considered distal-site neoplasms observed in rats to be "treatment-related," there is no evidence that they are "cobalt-related." The CDI considers a local increase in tissue cobalt levels to be necessary for the development of cobalt-related cancer. For example, following a two-week exposure of male and female rats to cobalt metal powder, both the cobalt levels in lung tissue and the incidence of alveolar/bronchiolar carcinoma showed exposure-response relationships. In contrast, following a two-week exposure of female rats to cobalt metal powder, the exposure-related increase in mononuclear-cell leukemia was not accompanied by an exposure-related increase in cobalt levels in the femur. Dr. Danzeisen stressed the importance of distinguishing between treatment-related and cobalt-related neoplasms, because of the implications for routes of exposure.

V. Peer Review of Draft RoC Monograph on Cobalt and Certain Cobalt Compounds

V.A.1 Cancer Evaluation Component

V.A.2 Properties and Human Exposure

V.A.2.1 Presentation on Properties and Human Exposure

Dr. Sanford Garner, ILS, presented an overview of the key information in the properties and human exposure sections of the draft monograph. Cobalt is a naturally occurring transition element present in several different metallic forms, and more than 100 cobalt compounds have been identified. The valence state of cobalt in compounds is most commonly +2 or +3. Cobalt compounds exist in many crystalline forms and colors, may be organic or inorganic, and vary in water solubility and bioaccessibility. In the draft monograph, "certain" cobalt compounds are defined as those that release cobalt ions *in vivo.* This class of compounds does not include vitamin B_{12}, because it does not release cobalt ions *in vivo.*

In vivo bioavailability of cobalt ions can be represented by the solubility of cobalt in artificial body fluids. Forms of cobalt of differing water solubility, including poorly soluble compounds, may show similarly high bioaccessibility in acidic gastric and lysosomal fluids. Poorly water-soluble compounds have generally lower bioaccessibility in neutral than in acidic fluids. Dr. Garner identified the representative cobalt forms for which carcinogenicity and genotoxicity are provided in the monograph.

The evidence suggests that a significant number of U.S. residents are exposed to cobalt, based on widespread use in numerous commercial, industrial, and military applications; high production volume of cobalt and several cobalt compounds (>1 million pounds per year); and biological monitoring data (cobalt levels in urine, blood, hair, and nails) in occupational and non-occupational populations. Uses, in decreasing order of extent, include metallurgical (in alloys, including superalloys and alloys used in medical devices), chemical (in pigments, driers, catalysts, adhesives, and animal diets), and in tungsten-carbide hard metals and bonded diamonds. Although use of cobalt in electronics and green energy accounted for less than 1% of

U.S. uses in 2012, this use is expected to increase, as about one third of cobalt use worldwide is for production of rechargeable batteries and other energy-related uses.

Humans are exposed to cobalt in the workplace, from medical procedures, from the environment, and from other sources. Urinary levels from exposure studies of the general public, environmental exposure, and stable hip implants overlap, range up to about 10 μg/l. Levels for occupational exposure and unstable hip implants reach up to 1,000 μg/l. Cobalt levels show generally similar patterns in urine, blood, hair, and nails.

The highest occupational exposures occur where powdered cobalt metal is used in production processes. Failure of cobalt-alloy hip implants through wear or corrosion by body fluids (in which there is inflammation of surrounding tissues) results in release and systemic transport of cobalt particles or ions, which can reach toxic levels in the serum. In 2013, environmental releases of cobalt and cobalt compounds from U.S. facilities totaled over 5 million pounds, and elevated urinary cobalt levels have been reported in people living near mining operations in Guatemala and Mexico. Environmental cobalt levels in the United States are in the ranges of parts per million in soil and sediment, parts per billion in water, and parts per trillion in air (primarily particulate cobalt); the average level in U.S. drinking water is about 2 ppb. Other sources of exposure to the general public include food, tobacco, and household cleaning products.

V.A.2.2 Peer-Review Comments on Properties and Human Exposure

Dr. William Jameson, first reviewer, stated that, except for the definition of “certain” cobalt compounds, these sections were clear and appeared to be technically correct. However, in defining “certain” cobalt compounds for the intended audience of the RoC, he suggested referring to “bioavailable” or “bioaccessible” cobalt ions, instead of cobalt ions released *in vivo.* He noted that in Table 1-1, “soluble” should be defined quantitatively. In Section 2.6, the reason for adding cobalt to beer should be stated.

Dr. Jameson agreed that the information in the draft monograph demonstrates significant exposure to cobalt and cobalt compounds in the United States. Exposure to cobalt or cobalt compounds in the electrochemical industry needs to be addressed in Section 2, to support the information on electrochemical workers in Section 4. Dr. Jameson noted that cobalt levels in U.S. workers and other U.S. populations can be inferred from biomonitoring of non-U.S. workers and others exposed to cobalt and cobalt compounds under similar exposure scenarios.

Dr. Robert Herrick, second reviewer, found these sections to be clear and well organized. He noted that on page 1, it is stated that the review does not include studies of cobalt alloys; however, Section 4 describes a study of workers in stainless steel and alloyed steel plants. He suggested clarifying the statement on page 1 concerning alloys. On page 2, the size ranges of nanoparticles and microparticles should be defined, and more detail should be provided on the criteria for satisfactory within-laboratory variability in bioaccessibility testing. In Section 2.3, information on methods for measurement of cobalt in air and on surfaces should be added. It would be helpful to add any available information on exposures associated with recycling of rechargeable batteries on page 6 and exposure information from NIOSH Health Hazard Evaluation reports in Section 2.4.

V.A.2.3 Panel Discussion on Properties and Human Exposure

Dr. Clark Lantz said that bioaccessibility data should be added for cobalt oxide (Co_3O_4). Dr. Anatoly Zhitkovich noted that inconsistencies in levels of cobalt in biological fluids between older and newer studies could be due to a significant improvement made in the mid 1980s to background correction for metals in atomic absorption spectroscopy. Dr. Howard said that if the discussion of the addition of cobalt to beer is expanded, the information should include when and where this practice was used. He suggested that discussion of the solubility of cobalt powders should take into account their large range of size and surface area.

With respect to inclusion of human studies on cobalt alloys, Dr. Lunn clarified that studies of workers involved in manufacturing of cobalt alloys were included if there were separate estimates for cobalt exposure. She said additional information, received from CDI, on the solubility of cobalt compounds would be considered in revising the draft monograph.

The Panel concurred with the statement that a significant number of persons living in the United States are exposed to cobalt and certain cobalt compounds.

V.A.3 Human Cancer Studies

V.A.3.1 Presentation on Human Cancer Studies

Dr. Pamela Schwingl, ILS, presented an overview of the key information in the draft monograph on studies of lung cancer, esophageal cancer, and other cancers in humans.

To assess their utility for informing the hazard evaluation, the studies were evaluated for potential bias in five domains (selection, exposure assessment, outcome assessment, confounding, and analysis and reporting) and for study sensitivity (ability to detect an effect, based on numbers of exposed and unexposed participants, evidence of substantial exposure during the appropriate exposure window, range of exposure levels or durations adequate for evaluation of exposure-response relationships, and sufficient follow-up time for detection of cancer). Judgments were made in each domain as to the level of concern about bias or sensitivity relative to an ideal study, and the domain-level judgments were integrated to rank the studies with respect to utility in broad categories.

Because lung cancer has a low survival rate, mortality is an adequate measure of incidence. Relevant potential confounding factors include smoking and occupational exposure to asbestos, chromium, nickel, and arsenic. Cobalt was studied in five different occupational cohorts: French electrochemical (cobalt production) workers (a mortality study and follow-up analysis), French hard-metal workers (a mortality study of 10 factories and a later study of the largest factory), Danish porcelain painters (an incidence study), French stainless and alloyed steel workers (a nested case-control analysis of mortality data), and Norwegian nickel refinery workers (a nested case-control analysis of incidence data).

All but one of the studies (nickel refinery workers) had small numbers of workers exposed to cobalt alone, only half the studies evaluated exposure-response relationships, and each study had problems with potential confounding by co-exposures. The study of nickel refinery workers was considered to have moderate utility, and the rest of the studies were considered to have moderate/low or low utility.

All studies except for the study of stainless and alloyed steel workers showed an elevated risk of lung cancer associated with cobalt exposure; most showed an approximate doubling of risk. However, alternative explanations for the results could not be excluded, because of the studies' limitations. In the follow-up study of cobalt production workers, dropping 1 exposed case reduced the standardized mortality ratio (SMR) from 4.66 to 1.16. The studies of hard-metal workers did not control for co-exposures other than tungsten carbide, suggesting that the odds ratio (OR) and SMR could be biased away from the null. In the porcelain painters, lung-cancer risk was elevated in both the exposed and the unexposed workers. In the study of stainless and alloyed steel workers, exposure misclassification likely biased the results towards the null, and the study's findings for other known lung carcinogens were negative. In the study of nickel refinery workers, adjusting for carcinogenic co-exposures (including nickel) in the continuous-exposure model decreased the OR from a positive association to less than one, and it was not possible to control for co-exposures in the categorical-exposure model. However, in one workshop where the cobalt concentration was three times the nickel concentration, the OR for lung cancer was elevated fivefold, with a significant trend for duration.

Two population-based case-control studies of esophageal cancer, in western Washington State and Ireland, based exposure assessment on cobalt levels in toenail clippings taken at the time of enrollment (about 6-1/2 months after diagnosis in the Washington State study). Relevant potential confounding factors included alcohol use and smoking; occupational co-exposures were not considered. Both studies were judged to be of low utility because of lack of information on cobalt levels prior to cancer diagnosis, meaning that temporality could not be established. They were also limited by the use of only one sample per person, as cobalt levels in toenails are not highly reproducible. Only the Washington State study found an elevated risk of esophageal cancer associated with cobalt exposure category. Dr. Schwingl noted that the cobalt levels differed by an order of magnitude between the two studies, and that soil levels of cobalt are much higher in western Washington State than in Ireland.

These same two studies also reported findings for other aerodigestive cancers and Barrett's esophagus (a precancerous condition). Significant exposure-response relationships were found for oral cavity cancer and Barrett's esophagus; however, study limitations precluded drawing conclusions on causality. The cohort study of French cobalt production workers also evaluated buccal cavity, pharyngeal, and laryngeal cancer combined, but the risk was not significantly elevated, based on 2 exposed cases.

Dr. John LaPres asked whether there is any evidence that tumor body burden affects cobalt levels in the body. Dr. Schwingl said changes in cobalt levels would result not from tumor formation itself, but from other correlates of cancer, such as weight loss or changes in diet.

V.A.3.2 Peer Review Comments on Human Cancer Studies

Dr. Marie-Elise Parent, first reviewer, stated that the section was very well written and clear, and the scientific information presented was accurate. Concerning the exclusion of studies of hip implants from the evaluation, she questioned whether the extent of exposure to cobalt was any less clear for failed hip implants than in the occupational studies retained for review. In fact, studies measuring cobalt in urine and hair found very high levels in patients with failed hip implants, suggesting prior exposure, and Figure 2-1 shows higher urinary cobalt levels among

unstable hip implant patients than in most occupational groups. She also noted that nearly all of the human studies reviewed were subject to strong potential confounding from other workplace exposures, and that collinearity of exposure to cobalt and other metals in some studies precluded separate analysis of cobalt exposure. She therefore questioned the *a priori* exclusion of hip implant studies based on unclear extent of exposure and potential confounding.

Dr. Parent stated that important issues in evaluating overall study quality were raised and adequately explained in the draft monograph, and she appreciated that the quality of the exposure assessment was given considerable weight in ranking the studies. However, it was unclear why the study of porcelain painters was ranked higher than the studies of electrochemical workers with respect to addressing potential confounding.

A more coherent picture of the exposure window represented in toenail samples would be helpful. It would also be helpful to have information on the exogenous cobalt exposure of cancer patients and controls. For the occupational cohort studies, discussion of the use of protective measures against cobalt exposure would be helpful. Even if this information was lacking in the studies, it may have been taken into consideration in creation of job exposure matrices or by using calendar years as a proxy for standard hygiene practices over time.

Dr. Parent said the findings from the human studies were well synthesized and interpreted. She suggested that the small number of independent study populations (not just their limited sample sizes) should be mentioned in the conclusion of the evaluation. She agreed that confounding by other carcinogens could not be ruled out and suggested that future studies should be conducted on occupational groups identified *a priori* as having less potential for confounding by known carcinogens.

Dr. Lisa DeRoo, second reviewer, stated that the section was clear, technically correct, and objectively presented and that the evaluation approach was clear and objective. She thought that the draft monograph did a good job in discussing the main limitations of the studies, which included the small numbers of exposed cases in most studies, the limited exposure information in some studies, and the problem of co-exposures, even in the study with the greatest utility. She echoed the questions concerning the relevant exposure period for cobalt levels in toenails, the effects of tumor formation on cobalt levels, and the rationale for excluding hip implant studies from the evaluation. She suggested adding more detail to the tables about how the healthy worker effect and confounding were addressed in the studies. Figure 4-1 should be identified as referring to lung cancer studies.

Dr. Herrick, third reviewer, said the information in the section was clear, technically correct, and objectively presented. However, he found the mix of information in the last column of Table 4-3 to be confusing, because of inconsistencies in how study strengths and weaknesses were identified. He disagreed with the overall conclusion that the evidence for carcinogenicity in humans was inadequate. Although the information on carcinogenicity is limited, he felt that two studies provided adequate evidence for lung cancer associated with cobalt exposure. In the 1987 study of French cobalt production workers, the SMR was significantly elevated among workers engaged exclusively in cobalt production. Although the study lacked information on smoking, the SMR for lung cancer in the cohort did not differ significantly from that of French males in the general population. Dr. Herrick questioned whether there was reason to think that

these workers smoked more than the general population. The study of Danish porcelain painters had no significant co-exposures. Although the study did not control for smoking, the reported smoking rates did not differ substantially between the exposed and referent groups and all unskilled women in Denmark. Despite the study's limitations, it found a significant excess of lung cancer among the exposed workers. Furthermore, it was not documented that the "unexposed" workers, who also showed an excess of lung cancer, were not exposed to cobalt, though not engaged in spraying the cobalt-containing material.

V.A.3.3 Panel Discussion of Human Cancer Studies

Dr. Zhitkovich agreed that the exclusion of cobalt-containing implants from the evaluation was problematic. He noted that chromium would be released from implants as noncarcinogenic Cr(III), not as the carcinogenic Cr(VI). Dr. McDiarmid concurred, noting that in implant patients, chromium levels are not high, and other investigators have not found Cr(VI).

Dr. Jameson said the information from the cancer studies in humans was clear and well written.

Dr. Tania Carréon-Valencia, NIOSH, did not concur that the studies of French cobalt production workers or Danish porcelain painters provided adequate evidence for lung carcinogenicity; though co-exposures were not a problem, the studies did not provide good estimates of cobalt exposure.

Dr. Parent noted that in the extended follow-up of the cobalt production workers cohort, removing 1 exposed case caused the association between cobalt exposure and lung cancer to collapse. She stressed that risk estimates based on small numbers of exposed cases are not robust.

Dr. Lunn explained that implants were excluded from the evaluation *a priori* because they represent a mixture of cobalt and chromium, and it was not until the occupational studies were evaluated in detail that the high degree of co-exposure became apparent.

Dr. LaPres noted that because the use of metal-on-metal hip implants in the United States is relatively recent, it may be too soon for cancer studies of hip implants to be useful. Several Panel members suggested that the draft monograph should better explain the *a priori* exclusion of these studies. Dr. McDiarmid said ceramic implants are also a concern, as the metal stem in the femur is also a source of cobalt exposure, and that excursions in blood cobalt levels are also seen with implants that have not failed. She suggested that NTP review studies of exposure to cobalt from hip implants that evaluated cancer outcomes. Dr. Zhitkovich noted that cobalt may act as a cancer promoter, making shorter-duration studies relevant, and that exposure to cobalt from implants is a major public concern. It was generally agreed that the available literature on hip implants would not change the conclusion that the data from human cancer studies are inadequate to evaluate the association between cobalt exposure and cancer.

V.A.3.4 Actions

Dr. Lantz moved, Dr. Jameson seconded, and the Panel voted (8 yes, 1 no, 0 abstentions) that the scientific information presented from human cancer studies supports the NTP's preliminary level of evidence conclusion of *inadequate evidence of carcinogenicity* of cobalt and certain cobalt compounds. Dr. Herrick voted no because he did not think the data were inadequate as

two studies without significant confounding from other exposures both found significant associations between cobalt exposure and lung cancer.

Dr. Jameson moved, Dr. Wise seconded, and the Panel agreed unanimously (9 yes, 0 no, 0 abstentions) that NTP should review the literature on human cancers and cobalt-containing joint replacements and convene another peer review if they identify any relevant data that might change the evaluation.

V.A.4 Studies of Cancer in Experimental Animals

V.A.4.1 Presentation on Studies of Cancer in Experimental Animals

Ms. Diane Spencer, DNTP, presented an overview of the key information in the draft monograph on studies in experimental animals. Seventeen studies of cobalt and cobalt compounds in rodents (mostly rats) were identified, including studies in cobalt metal and both water-soluble and poorly water-soluble cobalt compounds and exposure routes of inhalation or injection (subcutaneous, intraperitoneal, intramuscular, intrathoracic, or intrarenal).

The studies were evaluated on five elements of quality (study design/population, exposure conditions, outcome measurement and assessment, confounding, and analysis and reporting) and on sensitivity (ability to detect an effect, based on species and sex, number of animals tested, and study duration). These assessments were integrated in an assessment of overall study utility. All studies reviewed were considered to have utility for the evaluation. Studies with high utility were the two-year NTP carcinogenicity studies of cobalt metal and cobalt sulfate in rats and mice. The studies of low or moderate utility were limited mainly by low sensitivity.

In the NTP studies, statistically significant, dose-related increases in lung tumors (alveolar/bronchiolar adenoma and carcinoma) were observed in rats and mice of both sexes exposed to cobalt metal or cobalt sulfate by inhalation. In female rats exposed to cobalt sulfate, non-statistically significant increases in cystic keratinizing epithelioma, a benign neoplasm that can progress to squamous-cell carcinoma, were considered to be exposure-related because of their rarity; a single squamous-cell carcinoma also was observed in the high-dose group. Non-neoplastic effects included alveolar epithelial hyperplasia and granulomatous alveolar inflammation in all groups except for mice exposed to cobalt sulfate and alveolar histiocytic cell infiltration in the mice exposed to cobalt sulfate. In a study of cobalt(II) oxide administered by intratracheal instillation, lung neoplasms were increased in males (statistically significant) and in females (statistically non-significant). Male hamsters exposed to cobalt(II) oxide by inhalation showed pneumoconiosis, but no lung tumors; however, the study's sensitivity was limited by poor survival, and hamsters are considered a less sensitive model for detecting lung tumors than other rodents.

In the NTP inhalation exposure studies of cobalt metal, systemic effects also were observed, including significant increases in pancreatic islet-cell adenoma and carcinoma combined in male rats and mononuclear-cell leukemia in female rats. In male rats, findings for renal tubule adenoma and carcinoma combined were equivocal; however, this is a rare tumor, and a dose-related trend was observed. In rats of both sexes, the incidences of benign and malignant pheochromocytoma of the adrenal gland were increased in the NTP studies of cobalt metal and

cobalt sulfate; however, it was unclear whether this was a direct effect of cobalt exposure or an indirect response to lung damage. Cobalt metal and cobalt compounds (both water-soluble and poorly water-soluble) also induced injection-site tumors. Injection exposure could be relevant for humans, for example, for exposure from hip implants. These findings are considered supporting evidence for carcinogenicity, because of the consistency of tumor types, similar findings for different forms of cobalt, and evidence that the tumors were induced by cobalt and not just a reaction to the physical implant.

In regard to the finding of mononuclear-cell leukemia in female rats, Dr. Jameson noted that the historical control database for the F344/NTac rats used in the NTP study of cobalt metal was very small; he asked whether the results had been compared against the much larger historical control database for F344/N rats. Dr. Michael Pino said he believed that in some other NTP studies, the incidence of mononuclear-cell leukemia (which was slightly higher in inhalation than in oral exposure studies) was up to 52% in the F344/N historical controls; thus, the incidences in the NTP study of cobalt metal were slightly outside the historical control range for F344/N rats. Dr. Lunn said no later studies had been reported for F344/NTac rats. She noted that in addition to the increased incidence of mononuclear-cell leukemia, the NTP study found a decreased time to first tumor.

Dr. Danzeisen commented that cobalt(II) oxide, while poorly soluble in water, is highly soluble in lysosomal fluid.

V.A.4.2 Peer Review Comments on Studies of Cancer in Experimental Animals

Dr. Pino, first reviewer, stated that the scientific information presented in the section was clear, objectively stated, and technically correct except for a few minor items. He found the assessment of the utility of the animal studies to be systematic, transparent, objective, and clearly presented, especially in Appendix D.

Dr. Pino said that the strongest evidence for carcinogenicity is from the NTP bioassays of cobalt metal and cobalt sulfate. He agreed that these studies were very well conducted and of high utility for assessing carcinogenic potential. He agreed that the utility of the hamster inhalation study and the intratracheal instillation and injection studies in rats and mice was moderate to low, particularly because the purity of the test article was often inadequately characterized and the methods and results were inadequately reported. He agreed that the studies of injection at various anatomic sites provided supportive evidence for cobalt carcinogenicity and that the co-carcinogenicity studies were of low utility for assessing the carcinogenic potential of cobalt.

Dr. Pino suggested adding data for the extended evaluation of renal tubular adenoma, along with a brief explanation of the extended evaluation method. He also suggested adding the mass median aerodynamic diameter for aerosolized cobalt sulfate and cobalt oxide in the inhalation studies.

Dr. Pino said that although the NTP study of cobalt metal showed clear evidence of tumors in the lung and adrenal medulla, the findings of mononuclear-cell leukemia and pancreatic islet-cell tumors were difficult to interpret. The mononuclear-cell leukemia in female rats could not be clearly associated with cobalt because of the high background incidence of this tumor, the paucity of historical data for the F344/NTac rat, and the lack of dose response. The increased

incidence of pancreatic islet-cell tumors was observed in only one sex and species. Dr. Pino concluded that although the adrenal medullary and pancreatic islet-cell tumors were clearly treatment-related, it was not clear whether these were direct or indirect effects of cobalt exposure.

Dr. Lantz, second reviewer, found the information in the section to be clear and objectively presented. The summary tables provided information in an easy-to-access format and accurately reflected the literature. The approach to determining inclusion or exclusion of studies and evaluation of study quality and sensitivity was systematic and well defined, and he agreed with the assessment of study quality and sensitivity.

Dr. Lantz mentioned two apparent discrepancies between the information in the cited articles and the summary in the draft monograph. The characterization of particle and bulk samples in Hansen *et al.* (2006) should be checked for accuracy. Regarding Steinhoff and Mohr (1991), the statement that "in the low-dose group, only those with gross lesions were examined (histologically)" is inaccurate; the article states that the respiratory tract was examined histologically in all animals.

Dr. Lantz noted that poorly water-soluble yet bioaccessible compounds (such as cobalt oxide and sulfide) are less well studied in animals than metallic cobalt or cobalt sulfate. Although not all animal exposures to cobalt(II) oxide resulted in increases in tumors, the positive study results indicated that cobalt(II) oxide causes tumors, even at injection sites. The effects of cobalt sulfide were examined in one study, at a very low dose, and no positive results were reported. No long-term animal studies have examined the effects of exposure to other compounds, such as Co_3O_4. Dr. Lantz agreed with Dr. Pino that indirect effects of cobalt exposure could be involved in the occurrence of tumors at distal sites.

Dr. Jameson, third reviewer, said that the scientific information from cancer studies in experimental animals was objectively presented and mostly technically correct. In several areas, additional information on the studies' materials and methods should be provided. The approach and assessment of the animal carcinogenicity studies appeared to be systematic, transparent, objective, and, in most cases, clearly presented.

Dr. Jameson identified several points that needed clarification. Several studies were identified as having been excluded from the evaluation because they lacked concurrent controls, but were not included in Appendix D, and several studies included in the evaluation also lacked concurrent controls. The exclusion criteria appear inconsistent and should be clarified. The procedure for determining the denominator for tumor incidence and calculating the incidence should be clarified, as these appear to be inconsistent. It should be noted that the NTP study of cobalt metal in male rats is limited by a significant decrease in survival (which could account for the fact that leukemia was not observed in males). It also should be noted that the Steinhoff and Mohr (1991) study is limited by poor reporting and does not identify the vehicle(s) used in injection studies. Dr. Jameson concurred with the previous reviewers that the co-carcinogenicity studies provided little, if any, support for the co-carcinogenicity of cobalt compounds.

Dr. Lunn clarified that the evaluation excluded studies with no controls and included three studies that used non-concurrent controls. With respect to the tumors at distal sites, she noted

that the evaluation did not consider mechanistic data, but drew conclusions based on whether effects were treatment-related.

V.A.4.3 Panel Discussion of Studies of Cancer in Experimental Animals

Dr. Howard commented that injection exposure of animals is not similar to human exposure from hip implants; hip implants result in long-term, low-level exposure, whereas injection exposure results in shorter-duration exposure to a higher maximum cobalt concentration. Dr. Zhitkovich said exposures in experimental animals never realistically mimic human exposures; extrapolating from animal to human exposures is always an issue.

V.A.5 Disposition and Toxicokinetics and Mechanistic and Other Relevant Data

V.A.5.1 Presentation on Disposition and Toxicokinetics

Mr. Stanley Atwood, ILS, presented an overview of the key information in the draft monograph on disposition and toxicokinetics of cobalt and certain cobalt compounds. The primary routes of exposure are diet and inhalation; dermal exposure can occur but is important only in unusual circumstances.

Absorption of cobalt from the gastrointestinal (GI) tract in both humans and experimental animals is highly variable, ranging from practically none to almost complete. Recent toxicokinetic models use absorption figures of 20% to 45% for aqueous forms and 10% to 15% for solid forms. Absorption is about twice as high in women as in men, possibly because it is increased by iron deficiency, and is higher for water-soluble compounds than for water-insoluble compounds. Unabsorbed cobalt is excreted in the feces.

Airborne cobalt particles and water-soluble cobalt compounds are rapidly absorbed in the lungs, and cobalt concentrations in the blood and urine of workers are correlated with cobalt concentrations in air, especially of water-soluble forms. Large particles tend to deposit in the upper airways, are cleared by mucociliary action and swallowed, and may be excreted in the feces or solubilized and absorbed from the GI tract. Smaller particles may deposit in the bronchiolar and alveolar regions, where they may dissolve and be absorbed, be phagocytized by alveolar macrophages, or enter lung cells via endocytosis. Nanoparticles may translocate directly to the blood and lymph.

Cobalt is rapidly distributed to all tissues; in both humans and experimental animals, the highest concentrations generally are in liver and kidney. Stored cobalt does not significantly accumulate in the body with age. Because cobalt is extensively bound to plasma proteins and taken up by red blood cells, the free fraction in the blood is low (5% to 12%). Insoluble particles may be retained in the lungs and release cobalt over time. In both humans and experimental animals, much of absorbed cobalt is excreted within the first few days to a week after exposure. Multiphasic elimination of cobalt has been shown following intravenous administration to humans and inhalation, oral, and intravenous administration to experimental animals. In humans, about 40% is eliminated with a half-life of 6 to 12 hours, 50% with a half-life of 2 to 60 days, and 10% with a half-life of two years or more. Elimination is generally slower and retention the lungs longer in humans than in experimental animals.

V.A.5.2 Peer-Review Comments on Disposition and Toxicokinetics

Dr. Jameson, first reviewer, stated that the section was well written and concise. Species and sex differences in disposition include lower rates of translocation of cobalt from the lung to the blood in humans than in rodents, a longer whole-body half-life of cobalt in humans than in rodents, and higher GI absorption in women than in men, possibly reflecting iron status. In human lung cells *in vitro*, water-insoluble cobalt oxide particles are readily taken up through endocytosis and are partially solubilized at the low pH within lysosomes, while water-soluble cobalt salts enter cells via cellular transporters such as calcium channels or the divalent metal ion transporter. Skin absorption is closely related to the capacity of synthetic sweat to oxidize metallic cobalt powder to soluble cobalt ions. Uptake of cobalt by red blood cells is practically irreversible, because the ions bind to hemoglobin and are not extruded by the calcium pump. Toxicokinetic studies indicate multiphasic elimination following inhalation of cobalt particles or intravenous injection of cobalt chloride, with generally shorter elimination half-lives in experimental animals than in humans. Taken together, the information on disposition and toxicokinetics confirms the release of cobalt ions *in vivo*. Water-soluble cobalt compounds release ions into extracellular fluids, and poorly water-soluble cobalt compounds and cobalt particles and nanoparticles release cobalt ions intracellularly in lysosomes.

Dr. LaPres, second reviewer, concurred with Dr. Jameson and said the information on disposition and toxicokinetics was clear, technically correct, and objectively presented. He suggested that Table 3-1 should present both retention of the total dose of Co_3O_4 and retention relative to the amount remaining at 3 days after exposure, not just the latter.

V.A.5.3 Presentation on Mechanistic and Other Relevant Data

Mr. Atwood presented an overview of the key information in the draft monograph on mechanistic and other relevant data. Cobalt particles and ions have similar biological effects *in vitro* and *in vivo,* including cytotoxicity, inflammation, and formation of reactive oxygen species (ROS). Nanoparticles are generally more toxic than microparticles, and relatively water-soluble particles are more toxic than ions, which in turn are more toxic than poorly water-soluble particles. Nanoparticles and microparticles are more effective than ions in inducing ROS, and nanoparticles can activate neutrophils, which can generate ROS and proinflammatory cytokines. Cobalt particles must come in direct contact with a cell in order to induce biological effects.

In general, cobalt ions are responsible for most of the biological effects of cobalt and cobalt compounds, and differences in effects of exposure to different forms of cobalt are partially explained by differences in cellular uptake mechanisms. Although extracellular cobalt ions are more cytotoxic than poorly water-soluble cobalt particles, they result in similar intracellular concentrations of solubilized cobalt. While the ions are actively transported into the cells, the poorly soluble particles are taken up by endocytosis and dissolve in the lysosomes, which release cobalt ions into the cytoplasm.

Several mechanisms likely are involved in the biological effects of cobalt ions. Cobalt and certain cobalt compounds induce a similar spectrum of genotoxic and related effects, primarily clastogenic effects and DNA damage. It is generally believed that the genotoxic effects of cobalt are due to ROS and oxidative damage to DNA rather than direct interaction of cobalt with DNA.

The evidence for mutagenicity *in vitro* is mostly negative in bacteria and mixed in human and rodent cells. Cobalt also induces cell transformation in rodent cells *in vitro.* The evidence from *in vivo* studies is limited and mixed. Cobalt is known to inhibit DNA repair by inhibiting nucleotide excision repair and substituting for Zn(II) in zinc finger domains of DNA-repair proteins and transcription factors.

Cobalt in aqueous solutions can generate ROS, which can activate redox-sensitive transcription factors, such as nuclear factor κB (NF-κB) and activator protein-1 (AP-1), thus promoting tumor growth by dysregulation of cell growth, proliferation, and apoptosis. Evidence that cobalt and cobalt compounds cause oxidative stress or damage includes (1) overactivation of nuclear erythroid 2-related factor, which regulates genes involved in cellular antioxidant and anti-inflammatory defense, (2) increased sensitivity of knockout mice deficient in the DNA repair enzyme 8-oxoguanine-DNA glycosylase, (3) dose-dependent increases in ROS in human and animal cells *in vitro,* (4) evidence of oxidative damage to DNA in lung, liver, and kidney in rats exposed by injection, and (5) increased frequency of K-*ras* G to T transversion mutations in cobalt-induced lung tumors in rodents in the NTP studies.

Cobalt and cobalt compounds (including water-soluble cobalt salts, metal nanoparticles, and poorly water-soluble Co_3O_4) are well known to stabilize hypoxia-inducible factor 1α (HIF-1α), which regulates more than 100 hypoxia-responsive genes that promote cell survival under low-oxygen conditions. HIF-1α stabilization plays a major role in adaptation of cancer cells to hypoxia, and its overexpression and stabilization has been documented in more than 70% of human cancers.

The rationale for evaluating “cobalt and certain cobalt compounds” as a class is that for all cobalt forms tested, toxicity is attributed primarily to the cobalt ion, and that cobalt metal, water-soluble cobalt compounds, and poorly water-soluble cobalt compounds all release cobalt ions in biological fluids and have similar biological effects, including similar carcinogenic effects in rodents *in vivo.*

In response to a question from Dr. Danzeisen, Mr. Atwood clarified that endocytosis of Co_3O_4 particles was clathrin-mediated, and that the particles were around 400 nm. Dr. Danzeisen noted that the cobalt materials on the market tend to have larger particle sizes and would be expected to enter the cell via phagocytosis, rather than endocytosis.

V.A.5.4 Peer-Review Comments on Mechanistic and Other Relevant Data

Dr. Wise, first reviewer, found that the section was clear, technically correct, and objectively presented. However, he thought more context was needed. The mechanisms presented were those shown in the literature, but no information was provided on dose, exposure time, or the model systems used, thus implying that these mechanisms occur at all doses and exposure times across all model systems. Information on these factors would make it easier to evaluate the relevance or importance of the mechanisms. Synthesis is needed to put the mechanisms in context.

Some potential mechanisms, such as epigenetic changes, are not discussed. As written, the section seems to imply that other mechanisms are not relevant, when in fact the data may just be sparse, as epigenetics, for example, is a relatively new area. It should be clarified that the

mechanisms discussed are the ones for which data are available, not that all other mechanisms have been ruled out.

Dr. Wise noted a heavy reliance on nanoparticle data, which is scientifically problematic. The industrial uses of nanoparticles are based on the idea that chemicals act differently and have different properties on a nanoscale than on a microscale. It may be that their toxicological properties also differ. The section is written from the point of view that cobalt nanoparticles are simply smaller microparticles and thus represent cobalt. The *a priori* assumption in the nanotoxicology field is that the toxicology of nanoparticles may differ from that of microparticles; therefore, the argument must be made that they are acting the in same way as microparticles in order to justify including them in the analysis as representative of cobalt.

Regarding G to T transversions, which are often considered a characteristic mutation of oxidative damage, Dr. Wise suggested more discussion about whether this lesion might have had other causes, such as DNA-protein crosslinks. The draft monograph does not discuss how cobalt ions themselves might associate with DNA and cause lesions.

Dr. Wise found Appendix E to be clear, technical correct, and objectively presented. He suggested that apoptosis and transformation be discussed separately, as they are opposing outcomes. He suggested discussing cell death measures other than apoptosis as potential consequences of genotoxicity.

Dr. Zhitkovich, second reviewer, found the section to be generally well written. Regarding modes of action, he suggested modifying the accompanying figure by (1) deleting redox-sensitive transcription as an early event in carcinogenesis, (2) adding ‘avoidance of cell death’ as an early key event, and (3) clarifying whether oxidative damage should be added to the ROS mode of action.

He suggested removing the discussion of apoptosis, as it is a nonspecific marker of cell response to severe stress, and it is outdated to consider it as a marker of genotoxicity. He considered that the relevance to the evaluation of cobalt nanoparticles was clear from the draft monograph’s discussion of cobalt particles and cobalt ions, since the toxic entity is the cobalt(II) ion, which is released by cobalt nanoparticles.

Dr. Zhitkovich said the draft monograph overstated the evidence for mutagenicity of cobalt. Cobalt is clearly clastogenic *in vitro,* but only weakly mutagenic in mammalian cells and nonmutagenic in most bacterial mutagenicity assays. One proposed mechanism for cobalt’s clastogenicity is inhibition of DNA repair, resulting in a buildup of endogenous DNA damage. The second proposed mechanism is generation of ROS, which diffuse and damage DNA. The draft monograph cites studies reporting oxidative stress in cobalt-treated cells, but it is not clear which studies actually showed oxidative DNA damage. Dr. Zhitkovich suggested a more critical evaluation of the evidence for ROS as a mechanism of cobalt genotoxicity.

Dr. Zhitkovich said there is no question that cobalt acts as a chemical hypoxia mimic at low concentrations across all types of cells, and that hypoxia is extremely important in tumor development. The question is how this mechanism operates with cobalt. He noted that human patients with Von Hippel–Lindau syndrome develop cancer in multiple organs as a result of a genetic defect that results in upregulation of the hypoxia stress response. These patients

develop pheochromocytoma and adrenal gland, kidney, and pancreatic cancer. Thus, this human syndrome that mimics the effects of cobalt shows a pattern of cancers very consistent with what was observed in experimental animals exposed to cobalt.

Dr. Zhitkovich said the section on cell signaling and gene expression modulation contains a mixture of information, some of which should be moved to the section on hypoxic signaling. It is not adequately explained why activation of NF-κB and AP-1 could be involved in cancer. The section identifies cellular stress responses activated by cobalt. However, stress responses are generally cellular defense mechanisms, and they are not necessarily important for cancer development. For example, activation of the p53 tumor suppressor prevents cancer. He concurred with Dr. Wise that the section needs to be rewritten and could include the discussion of epigenetic mechanisms.

Dr. Zhitkovich said the synthesis of information on mechanistic and other relevant data accurately described what is in the literature. He agreed with the basis for recognizing a class of cobalt compounds that release cobalt(II) ion and operate by a similar mechanism, and with the potential roles of genotoxicity (i.e., clastogenicity) and hypoxic signaling, though not necessarily oxidative stress (since cobalt is not a very strongly redox-active metal). He noted that in cases where the mechanism of DNA damage is not clear, people tend to default to oxidative stress as an explanation.

Dr. LaPres, third reviewer, found the information on mechanistic data to be clear, technically correct, and objectively presented. He thought the topic of apoptosis should be retained in the context of an added discussion of mitochondrial dysfunction. Cobalt's interference with intracellular iron transport could result in mitochondrial dysfunction, resulting in the formation of ROS. In addition, ROS should be mentioned as a third putative mechanism for stabilization of HIFs; it has been suggested that an increase in ROS would alter the iron redox chemistry and the hydroxylases that regulate HIF stability. Dr. LaPres stressed the importance of considering cobalt's effects on all iron-containing enzymes—not just the prolyl hydroxylases that affect HIF stability, but also those that affect collagen formation or regulate HIF transcription. He noted that cobalt stabilizes not only HIF-1α but all three mammalian HIFs.

Dr. Zhitkovich agreed that HIF-2 should be discussed. He noted that HIF activation by cobalt has been reported to be ROS-independent. Dr. LaPres clarified that he had mentioned the proposed role of ROS in HIF stabilization for completeness, and that is has been proposed that HIF activation is instead limited by oxygen availability.

V.A.5.5 Panel Discussion on Disposition and Toxicokinetics and Mechanistic and Other Relevant Data

Dr. Bucher asked the Panel to comment on the mechanistic data in terms of whether the proposed mechanisms are relevant to human cancer and whether the evidence on mechanisms is strong enough to be mentioned in the RoC listing as supporting evidence for carcinogenicity.

The Panel concurred that the mechanistic data are relevant to humans and supported grouping cobalt and certain cobalt compounds that release cobalt ion *in vivo* as a class. Dr. Jameson said that he considered all of the mechanisms reviewed to be relevant to humans. In Figure 6-1, he

suggested changing “poorly soluble cobalt particles” to “poorly soluble cobalt compounds and cobalt metal particles.”

V.A.6 Overall Cancer Evaluation and Preliminary Listing Recommendation

V.A.6.1 Peer-Review Comments on Integration of Animal, Human, and Mechanistic Data

Dr. Lantz, first reviewer, agreed that the human studies in the current literature are inadequate to determine the carcinogenicity of cobalt and cobalt compounds. The evaluation clearly defines “certain cobalt compounds” as those compounds that can release cobalt ions *in vivo*. The NTP inhalation studies clearly indicate that both cobalt metal and cobalt sulfate increase the incidence of lung tumors in both mice and rats. Injection-site tumors have also been shown to occur in animals exposed to cobalt metal and cobalt chloride. These compounds are highly bioaccessible either in aqueous solutions or acidic environments.

Based on the definition of “certain cobalt compounds,” inclusion in the RoC classification will depend on the solubility of cobalt compounds *in vivo.* Although cobalt(II) oxide (CoO) is insoluble in aqueous solutions, it is highly soluble in acidic lysosomal environments and is easily classified with the more soluble compounds under those conditions. Animal studies of cobalt oxide have shown that long-term exposures can produce carcinogenic effects, and the cytotoxicity and genotoxicity of cobalt oxide and cobalt chloride are similar at similar intracellular levels of solubilized cobalt, indicating that the adverse effects result from the dissolution of cobalt from cobalt oxide.

Dr. Lantz said more problematic are cobalt particles with very low solubility, even in acidic lysosomal conditions, such as Co_3O_4. No animal studies have looked at the carcinogenicity of this compound. However, *in vitro* studies indicate that the level of cytotoxicity in lung airway epithelial cells exposed to Co_3O_4 is related to the solubilized cobalt. A major mode of action for Co_3O_4 nanoparticles is generation of ROS with accompanying oxidative DNA damage. This is consistent with the mechanisms of action identified for cobalt exposures and the inclusion of Co_3O_4 under the definition of “certain cobalt compounds.” In Table 7-1, CoO and Co_3O_4 are grouped under “cobalt oxide.” Because of their large difference in solubility, Dr. Lantz suggested listing these two compounds separately in the table.

Dr. DeRoo, second reviewer, suggested that in the section on human cancer studies, the small numbers of exposed cases should be noted as a reason for the inadequacy of the studies.

Dr. Wise, third reviewer, said that the definition of “certain cobalt compounds” was not clear enough. He suggested eliminating the word “certain” and calling the class “cobalt and cobalt compounds that can release cobalt ions *in vivo*.” He thought the main points of the synthesis, including justification for listing cobalt and cobalt compounds as a class, could be made more clearly. He suggested reorganizing the section to improve the flow and clarify the synthesis.

V.A.6.2 Panel Discussion on the Integration of Animal, Human, and Mechanistic Data

Dr. McDiarmid asked whether the Panel agreed with the designation of the class of substances as being based on the release of cobalt ions. The Panel recommended using the definition of

"certain cobalt compounds," i.e., "cobalt compounds that release cobalt ions *in vivo*" in the listing rather than the word "certain." The listing would be "cobalt and cobalt compounds that release cobalt ions *in vivo*." Dr. Parent noted that some substances that release cobalt ions, such as cobalt alloys, were excluded from the review, and asked whether "certain" was meant to exclude these substances. Dr. Bucher said that the term was meant to include all compounds that release cobalt ions. The Panel generally agreed that the name of the class should be more descriptive, identifying the release of cobalt ions *in vivo* as its defining characteristic.

Dr. McDiarmid asked whether the Panel had any additional discussion on the CDI's comments. Regarding the distal tumor sites, it was clarified that regardless of whether the mode of action is direct or indirect, treatment-related tumors are considered evidence of carcinogenicity.

V.A.6.3 Actions

Dr. Jameson moved, Dr. LaPres seconded, and the Panel agreed unanimously (9 yes, 0 no, 0 abstentions) that the scientific information presented from studies in experimental animals supports the NTP's preliminary level of evidence conclusion of *sufficient evidence of carcinogenicity* of cobalt and cobalt compounds that release cobalt ions *in vivo*. This is based on increased incidences of malignant and/or combined malignant and benign neoplasms induced in rodents by different forms of cobalt in inhalation and injection studies.

Dr. Wise moved, Dr. Herrick seconded, and the Panel agreed unanimously (9 yes, 0 no, 0 abstentions) with the NTP's preliminary policy decision to list "cobalt and cobalt compounds that release cobalt ions *in vivo*" in the RoC as *reasonably anticipated to be human carcinogens* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting data from studies on mechanisms of carcinogenesis.

V.B. Draft RoC Substance Profile

Ms. Spencer summarized the contents of the substance profile and the NTP's preliminary conclusions concerning cobalt and certain cobalt compounds.

Dr. Herrick, first reviewer, said the draft substance profile sections on properties, use, production, exposure, and regulations and guidelines were well written. With respect to the section on cancer studies in humans, Dr. Herrick referenced his previous comments on the evidence from human studies. In discussion, it was suggested that in the profile, the "other limitations" of the cancer studies in humans be specified, and that it be noted that the results of most of the human studies are consistent with the other evidence for carcinogenicity.

Dr. Parent, second reviewer, suggested that the profile mention which cobalt-containing substances were not included in the evaluation.

Dr. Pino, third reviewer, said that the information presented regarding cancer studies in experimental animals was generally clear, technically correct, and objectively stated, and that the key information was for the most part adequately highlighted. He suggested adding a statement that the tumors observed at distal sites, especially the adrenal gland tumors, could be due to indirect mechanisms. In the statement on page II-5 suggesting that cobalt metal is more carcinogenic than cobalt sulfate at a similar cobalt concentration (low dose of cobalt metal and high dose of cobalt sulfate), Dr. Pino suggested deleting the reference to the extent of systemic

lesions. He noted that the incidence of adrenal gland tumors was similar between these two groups. Although mononuclear-cell leukemia was seen only with cobalt metal, the incidence was similar across all cobalt-exposed groups and therefore difficult to interpret. Also, since the cobalt metal study used a different rat substrain, at least part of the difference in the results for both the lung and systemic tumors could be due to differences in substrain sensitivity.

Dr. Zhitkovich, fourth reviewer, said the section on mechanisms of carcinogenesis accurately summarized the information from the draft monograph section on mechanistic and other relevant effects, but had the same weaknesses. He repeated his previous suggestions to clarify the discussion of modes of action, recommending removal of the discussion of speculative or less-well-established mechanisms; clarifying that cobalt causes chromosome damage in mammalian cells, but does not appear to be mutagenic; and revising the text and figure to show the correct sequence of events.

Dr. Wise said the section on mechanisms of carcinogenesis was written too definitively; he suggested starting the section with a statement that the exact mechanism of cobalt carcinogenesis is unknown.

Dr. Howard expressed concern, from a public health communication aspect, that the exclusion of vitamin B_{12} from the class of carcinogenic cobalt compounds needed greater emphasis. Dr. Zhitkovich noted that this applies to all cobalt ingested in the diet, not just vitamin B_{12}. Dr. McDiarmid said the issue was a matter for experts in health risk communications.

Dr. Carréon-Valencia said the draft substance profile was well written and agreed with the peer-review comments on the limitations of the human cancer studies.

VI. Closing Remarks on Draft RoC Monograph

Dr. Jameson commended the NTP and their contract support staff for their work on the draft monograph and thanked Dr. McDiarmid for chairing the meeting. Drs. McDiarmid and Bucher thanked the Panel for their thoughtful and detailed review.

The meeting was adjourned at 3:48 p.m.

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Dr. Melissa McDiarmid

Chair, Peer-Review Panel

10/30/2015

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Date