National Toxicology Program Response to the Peer-Review Report

Peer Review of the Draft Report on Carcinogens Monograph on Cobalt

Public Meeting
July 22, 2015

National Institute of Environmental Health Sciences

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Introduction

The NTP convened an *ad hoc* scientific panel ("Panel") to peer review the *Draft Report on Carcinogens (RoC) Monograph on Cobalt and Certain Cobalt Compounds* at a public meeting held July 22, 2015, at the National Institute of Environmental Health Sciences, Research Triangle Park, NC (information on the meeting is available at http://ntp.niehs.nih.gov/go/38854). A draft RoC monograph consists of a cancer hazard evaluation component and a substance profile. The Panel had a two-fold charge:

1. To comment on the draft cancer hazard evaluation component, specifically, whether it was 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 RoC listing criteria.
2. To comment on the draft substance profile, specifically, whether the scientific justification presented in the substance profile supports the NTP's preliminary policy decision on the RoC listing status of cobalt and certain cobalt compounds.

The Panel was asked to vote on the following:

1. Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from human cancer studies of cobalt and certain cobalt compounds.
2. Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from experimental animal studies of cobalt and certain cobalt compounds.
3. Whether the scientific evidence supports the NTP's preliminary listing decision for cobalt and certain cobalt compounds in the RoC.

The Panel's peer-review comments were captured in the *Peer Review of the Draft Report on Carcinogens Monograph on Cobalt and Certain Cobalt Compounds* ("Peer-Review Report"). Per the process for preparation of the RoC, the NTP prepares a response to the Peer-Review Report and posts it on the RoC website (http://ntp.niehs.nih.gov/go/38854). The NTP's response addresses the Panel's (1) recommendations concerning NTP's draft conclusions and (2) scientific and technical peer-review comments related to identifying scientific issues and improving the technical accuracy, clarity, and objectivity of the monograph.

The NTP carefully reviewed and considered the Peer-Review Report in revising the draft monograph. The revised draft RoC monograph[1] will be shared with the public and the NTP Board of Scientific Counselors (BSC) at their public meeting on December 1-2, 2015, and finalized following the meeting.

[1]Available at http://ntp.niehs.nih.gov/go/730697.

Cobalt Peer-Review Panel[2]

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[2]The selection of panel members and conduct of the peer review were performed in accordance with the Federal Advisory Committee Act and Federal policies and regulations. The panel members served as independent scientists and not as representatives of any organization, company, or governmental agency.

Cobalt Panel Recommendations and NTP Response

Panel's recommendation on NTP conclusions and NTP response

NTP's preliminary listing decision for cobalt in the RoC

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 Report on Carcinogens 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. 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 therefore be "cobalt and cobalt compounds that release cobalt ions *in vivo*."

Mechanistic data and listing as a class

The Panel concurred that the mechanistic data are relevant to humans and supported grouping 'cobalt and cobalt compounds that release cobalt ions *in vivo*' as a class.

Level of evidence from studies in experimental animals

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.

Level of evidence from studies in humans

The Panel agreed (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.

Exposure to cobalt and cobalt compounds that release cobalt in vivo

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.

NTP Response: NTP concurs with the Panel that cobalt and cobalt compounds that release cobalt ions *in vivo* should be listed 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

Scientific and Technical Peer-Review Comments

The Panel provided scientific and technical comments that addressed issues related to the cancer hazard evaluation and for improving the clarity and completeness of the *Draft RoC Monograph on Cobalt*. The specific comments and NTP responses to those comments are discussed below; they are organized by the type of evidence stream (e.g., properties and human exposure, human cancer studies, mechanistic studies).

Comments and NTP's response on properties and human exposure

Panel Comments:

The majority of the Panel's comments were requests for adding information on properties and human exposure to cobalt.

- Comments on properties were primarily requests for more information on solubility testing of cobalt compounds including adding bioelution data for a greater number of cobalt compounds (if available), adding a discussion of the reliability of testing methods, and providing more details on physical properties of the tested cobalt compounds.
- Comments on human exposure were primarily to add more information on occupational exposure including information from other sources and the United States NIOSH Health Hazard Evaluations (HHE) reports on other industries (electrical chemical industry and rechargeable battery recycling) and on methods used to measure cobalt in the workplace.

NTP Response: NTP concurs with these comments and has added the information requested by the Panel. Newly acquired solubility data in gastric and lysosomal fluids for eight additional representative cobalt compounds as well as physical characteristics (e.g., size ranges of cobalt nanoparticles and microparticles) data for all the representative cobalt were added to Tables 1-1 (Section 1.2, Water solubility and bioaccessibility) and solubility data on other cobalt compounds were added to Table B-1 (see Appendix B.1, Chemical properties). A discussion of the reliability of bioaccessibility methods, as well as the improvements to these methods since the 1980s, was added to Section 1.2. In the draft RoC monograph on cobalt, only non-U.S. occupational data were available, thus the HHE reports on cobalt exposure in a variety of U.S. cobalt industries is an important asset to the revised RoC monograph. These data, as well as biomonitoring and environmental monitoring data in the cobalt production and recycling industries, were added to Section 2.5 (Characterization of exposure in the workplace) and Tables 2-5 (Section 2.5) and Table B-2 (see Appendix B.2, Exposure). A new section was created to discuss the recycling industry in general, and information on analytical methods used to measure exposure was added to Section 2.4 (Biomonitoring and environmental monitoring for cobalt).

Comments and NTP's response on the human cancer hazard evaluation

Panel Comments:

The Panel raised the following comments, which were mostly related to the interpretation of evidence from two cohort studies and issues related to evaluation of the biomarkers used in the case-control studies.

- One reviewer thought the evidence for carcinogenicity from the human studies was limited based on increased risk of lung cancer in two cohort studies (electrochemical and porcelain workers) where confounding from other occupational carcinogens was not a concern.

NTP Response: NTP agrees with the majority of the Panel members that the evidence for carcinogenicity from studies in humans is inadequate to evaluate rather than limited. Although both cohort studies reported a two-fold increased risk of lung cancer among cobalt-exposed workers and potential confounding from occupational co-exposures from other

metals is less of a concern, other weaknesses in the studies limited the confidence in the findings. There were only four exposed lung cancer cases in the electrochemical cohort study[3] and lung cancer risk was not increased in an update/reanalysis of the cohort[4]. In the study of porcelain workers, a two-fold increase in lung cancer was also observed among the "unexposed" workers[5].

- Provide information on the exposure window represented by the toenail samples in the case-control studies and potential effects of tumor formation on cobalt levels in toenails in the monograph.

NTP Response: NTP concurs with the Panel that information related to the exposure window of cobalt in toenail samples and effects of tumor formation on cobalt levels to the human cancer is important for understanding its conclusions about the population-based case-control studies using this biomarker of exposure. Relevant information from the clinical studies of cobalt levels in tumor tissues from cancer patients and controls (described in Appendix C.2.3, Information bias: Exposure assessment and disease endpoints) was integrated into Section 4.3, Case-control studies. Overall, this additional information supports the draft monograph conclusions that the data from the two case-control studies (Rogers *et al*. 1993, O'Rorke *et al.* 2012)[6] were inadequate to evaluate the relationship between exposure to cobalt and esophageal cancers because of concerns about the exposure window for cobalt represented in the toenail clippings. Toenail clippings likely reflect an integrated exposure that occurred 12 to 18 months prior to clipping and toenail samples were collected after cancer diagnosis in these studies. Many factors (including disease) can affect nail growth and metal deposition. The available studies that evaluated cobalt levels in tumor tissue and cancer stage (lung or laryngeal) are conflicting, thus it unclear whether the cancer process can affect cobalt levels in toenails (Benderli-Cihan *et al.* 2011; Kuo *et al.* 2006, Klatka *et al.* 2011).[7] The Rogers *et al*. study, as stated in the draft monograph, did not find differences in cancer risk stratified by tumor stage or time of diagnosis, which helps reduce concerns about reverse causality albeit concerns on the adequacy of the exposure window still remain.

Comments and NTP's response on the experimental animal hazard evaluation

The Panel made several comments requesting additional information for completeness and regarding scientific issues.

[3]Mur JM, Moulin JJ, Charruyer-Seinerra MP, Lafitte J. 1987. A cohort mortality study among cobalt and sodium workers in an electrochemical plant. *Am J Ind Med* 11(1): 75-81.
[4]Moulin JJ, Wild P, Mur JM, Fournier-Betz M, Mercier-Gallay M. 1993. A mortality study of cobalt production workers: An extension of the follow-up. *Am J Ind Med* 23(2): 281-288.
[5]Tuchsen F, Jensen MV, Villadsen E, Lynge E. 1996. Incidence of lung cancer among cobalt-exposed women. *Scand J Work Environ Health* 22(6): 444-450.
[6]Rogers MA, Thomas DB, Davis S, Vaughan TL, Nevissi AE. 1993. A case-control study of element levels and cancer of the upper aerodigestive tract. Cancer Epidemiol Biomarkers Prev 2(4): 305-312; O'Rorke MA, Cantwell MM, Abnet CC, Brockman AJ, Murray LJ, Group FS. 2012. Toenail trace element status and risk of Barrett's oesophagus and oesophageal adenocarcinoma: Results from the FINBAR study. *Int J Cancer* 131(8): 1882-1891.
[7]Benderli Cihan Y, Öztürk Yildirim S. 2011. A discriminant analysis of trace elements in scalp hair of healthy controls and stage-IIIB non-small cell lung cancer (NSCLC) patients. *Biol Trace Elem Res* 144(1-3): 272-294; Kuo CY, Wong RH, Lin JY, Lai JC, Lee H. 2006. Accumulation of chromium and nickel metals in lung tumors from lung cancer patients in Taiwan. *J Toxicol Environ Health A* 69(14): 1337-1344; Klatka J, Remer M, Dobrowolski R, Pietruszewska W, Trojanowska A, Siwiec H, Charytanowicz M. 2011. The content of cadmium, cobalt and nickel in laryngeal carcinoma. *Arch Med Sci* 7(3): 517-522.

- Most of the Panel comments were related to clarification or request that additional information be added to tables and/or text regarding vehicle and delivery methods, particle sizes and characteristics of the cobalt species, pathology procedures, age of the animal at start of the studies, and numbers of animals used to calculate tumor incidence or at sacrifice.

NTP Response: NTP has added information and has added, clarified, or corrected information (listed above) to Tables 5-3 to 5-5.

- Some reviewers stated that the occurrence of tumors sites at distal sites such as the pancreatic islet cell tumors and mononuclear-cell leukemia were more difficult to interpret and could be due to indirect rather than direct effects of cobalt. One reviewer also commented that the evidence from the mononuclear cell tumors was also limited by the lack of dose-response relationship and low numbers of historical controls.

NTP Response: NTP believes that the pancreatic islet cell tumors and mononuclear-cell leukemia are related to exposure to cobalt regardless of the mechanism (direct or indirect). The incidence of mononuclear cell leukemia (adjusted rates 59% to 62%) in females at all doses was at least 50% higher than the highest value of the range of the concurrent (adjusted rate 36%) or historical controls (38%) from all routes of exposure. In addition, time to first tumor was shorter in cobalt-exposed animals (117 to 590 days) compared to the concurrent control (663 days) albeit there was no pattern of decreasing duration with increasing dose and because of the limited historical control database, it is not known how much time to first tumor in untreated animals varies across studies.

- One reviewer noted that the study by Shabaan *et al.* (1977)[8] found that subcutaneous injection of a soluble cobalt compound, cobalt chloride caused fibrosarcomas in rats, which argues against the hypothesis that tumors in injection studies are due to physical properties of the material rather than a cobalt-specific effect.

NTP Response: NTP agrees that this study provides additional evidence to support NTP conclusions that the findings from injection-site tumors provide supporting evidence for the carcinogenicity of cobalt in experimental animals. The information has been incorporated into Section 5.3, which also discusses the findings from the study by Hansen *et al.* (2006)[9] showing that compounds that had similar physical characteristics (surface area to volume ratio) as cobalt metal did not cause injection-site tumors whereas cobalt did, which also supports the relevance of the injection-site tumors in the cancer hazard evaluation.

Comments and NTP's response on the genotoxicity, mechanistic, and other relevant data

Mechanistic and other relevant data is captured in Section 6 and Appendix E. The Panel commented on the proposed schematic (Figure 6-1) of the major modes of action discussed in the monograph as well the individual modes of actions, which are genotoxicity/inhibition of

[8]Shabaan AA, Marks V, Lancaster MC, Dufeu GN. 1977. Fibrosarcomas induced by cobalt chloride ($CoCl_2$) in rats. *Lab Anim* 11(1): 43-46.

[9]Hansen T, Clermont G, Alves A, Eloy R, Brochhausen C, Boutrand JP, Gatti AM, Kirkpatrick CJ. 2006. Biological tolerance of different materials in bulk and nanoparticulate form in a rat model: sarcoma development by nanoparticles. *J R Soc Interface* 3(11): 767-775.

DNA repair, reactive oxygen species (ROS) and HIF-1α stabilization. The figure also identifies early and late events that least to tumor formation.

Panel Comments:

- *Overall schematic*: One reviewer suggested changes in the schematic (and related discussion) of the proposed modes of action (MOAs) of cobalt carcinogenicity including (1) changing one of the MOAs, increase in ROS, to increase in ROS and oxidative damage, (2) deleting redox-sensitive transcription factors as an early event, and (3) adding avoidance of cell death, which permits accumulation of critical cancer-promoting genetic changes, as an early key event.
- *Genotoxicity:* The Panel noted that although cobalt is clearly clastogenic, the evidence for mutagenicity of cobalt was overstated in the monograph. They did not think cell transformation should be discussed in the genotoxicity and other relevant data section or appendices.
- *ROS:* One reviewer thought the monograph should discuss whether studies actually show oxidative DNA damage and provide a more critical evaluation or ROS as a mechanism for cobalt genotoxicity.

NTP Response: NTP revised Figure 6-1 according to the Panel's recommendations. The genotoxicity text and Appendix E have been revised to emphasize that cobalt primarily induces clastogenic effects and clarify that the mutagenicity data is weak. Section 6.2.1 (now called, Genotoxicity, DNA repair and other key events) was reorganized and expanded to help clarify the relationship between ROS and genotoxicity, and to discuss key events related to genomic instability and malignant transformation. This include a discussion of a study (Green *et al.* 2013)[10] which found that cobalt, via its interaction with p53, can increase resistance to apoptosis (i.e., avoidance of cell death that the Panel identified as an early effect), which can facilitate cell survival and expansion of damaged cells (e.g., cells with cobalt–induced chromosomal damage). Unrepaired genotoxicity can contribute to genomic instability and accumulation of critical mutations, leading to cellular transformation and proliferation. The expanded section also discusses studies finding that cobalt induced cellular transformation. Although cellular transformation assays do not measure genotoxicity *per se*, there is some evidence to suggest that ROS production or decreases in DNA repair of oxidative DNA damage, both of which can cause genotoxicity, play a role in cobalt-induced cellular transformation, The section on oxidative stress (6.2.2) was restructured to emphasize the available data on oxidative DNA damage.

- *HIF activation*: The Panel recommended that the monograph should note other HIFs in addition to HIF-1 and that the hypoxia stress response is analogous to the Von Hippel-Lindau syndrome (VHL). One reviewer thought the monograph should note that ROS should be noted as a putative mechanism for HIF-1α stabilization whereas another reviewer thought that HIF activation by cobalt has been reported to be ROS-independent and is limited by oxygen availability.

[10]Green SE, Luczak MW, Morse JL, DeLoughery Z, Zhitkovich A. 2013. Uptake, p53 pathway activation, and cytotoxic responses for Co(II) and Ni(II) in human lung cells: implications for carcinogenicity. *Toxicol Sci* 136(2): 467-477.

NTP Response: Information on HIF-1α stabilization, including its relationship to VHL, and discussion of other HIFs was added to Section 6.2.3 (HIF stabilization and hypoxia mimicry). Several studies have suggested that oxidative stress does not appear to be a primary mechanism of cobalt-induced HIF activation (Salnikow *et al.* (2000),[11] Nyga *et al.* (2015).

- *Cellular stress:* The reviewers noted that apoptosis is a non-specific response to severe stress and not a marker of genotoxicity, and should be removed from that section. One reviewer thought apoptosis should be removed from the entire monograph whereas other reviewers thought it should be retained but the discussion needed clarification and provision of context to carcinogenicity. The reviewers also recommended deleting other information on cell signaling and gene expression that was more related to cellular stresses than carcinogenicity.

  NTP Response: The monograph was revised to add a new section that integrates issues across these comments with other information in the monograph. This section discusses biological effects that may or may not be related to carcinogenicity, such as cobalt-induced respiratory or inflammatory adverse effects observed in humans and experimental animals (which was moved from other sections of the documents) and is important for evaluating compounds as a class. Studies of apoptosis have been moved from the genotoxicity section to this section as they may relate to lung injury. Information on cell signaling and gene expression that was not relevant for the evaluation of carcinogenicity or listing as a class was deleted.

Other Panel comments were related to weighing the importance of the mechanistic data or identifying other potential mechanisms (those not emphasized or discussed in the draft monograph).

Panel Comments:

- One reviewer thought that more context (such as dose used, exposure time, and model system) was needed to evaluate the relevance or importance of the mechanisms and that the monograph should clarify whether other mechanisms (such as epigenetics) may be relevant.
- Another reviewer noted that cobalt's interference with intracellular iron transport could result in mitochondrial dysfunction, resulting in the formation of ROS. He thought apoptosis could be discussed in relationship to mitochondrial dysfunction.

NTP Response: The purpose of the mechanistic section of the monograph is to provide information regarding whether the proposed mechanisms are relevant to human cancer and related to grouping cobalt and certain cobalt compounds as a class. 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. It is beyond the scope of the monograph to conduct a formal MOA evaluation or identify all potential, less established MOAs. NTP briefly acknowledged and listed the potential for other biological responses or modes of

[11]Salnikow K, Su W, Blagosklonny MV, Costa M. 2000. Carcinogenic metals induce hypoxia-inducible factor-stimulated transcription by reactive oxygen species-independent mechanism. *Cancer Res* 60(13): 3375-3378.

action such as epigenetics and disruption of signaling pathways), but in general felt that these studies were too sparse and speculative for inclusion in the monograph (see Section 6.2, Proposed mode of action of cobalt carcinogenicity). Section 6.2.3 (HIF stabilization and hypoxia mimicry) of the revised monograph briefly discusses studies related to cobalt-induced mitochondrial toxicity, ROS, and hypoxia-induced transcription. These studies reported that lysosomes rather than mitochondria were the source of ROS (Pourahmad *et al.* 2003) and that cobalt activates hypoxia-induced transcription via a mitochondria-independent mechanism (Karovic *et al.* 2007, Chandel *et al.*1998).

Comments and NTP's response on the overall evaluation and substance profile

- One reviewer noted that although there were no animal studies on Co_3O_4 nanoparticles, the mode of action for this compound is the generation of reactive oxygen species with accompanying oxidative damage and is consistent with the mode of action for the other compounds and thus should be included in the proposed class of cobalt and cobalt compounds. He recommended discussing the data for the chemical and biological properties and effects for CoO and Co_3O_4 separately in Table 7.1.
- In general, the comments on the draft substance profile were on points made for the cancer evaluation component (such as the Figure on the mechanisms of carcinogenicity).

NTP Response: The NTP concurs with the Panel comments that the mechanistic and biological effects data supports the inclusion of Co_3O_4 in the class of cobalt compounds that release ions *in vivo,* and has discussed this data, specifically for CoO and Co_3O_4, in the text accompanying Table 7-1. Although Co_3O_4 is not very soluble in artificial fluids (gastric and lysosomal), an *vitro* study of bioaccessibility of Co_3O_4 and a soluble cobalt compound (cobalt chloride, $CoCl_2$) in human lung cells found that intracellular concentrations of solubilized cobalt ions were similar for both compounds, suggesting that Co_3O_4 would release cobalt ions *in vivo* (Ortega *et al*. 2014)[12]. The substance profile was revised to captured the applicable Panel comments on the cancer hazard evaluation component that are on issues or text similar to that in the substance profile.

New Information

Both the Panel and the public made comments that were related to potentially including a large database of studies that were not reported or available at the release of the draft monographs.

Panel recommendations on hip implants

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. Some members of the Panel thought this was a small literature and would not be too burdensome to review.

[12]Ortega R, Bresson C, Darolles C, Gautier C, Roudeau S, Perrin L, Janin M, Floriani M, Aloin V, Carmona A, Malard V. 2014. Low-solubility particles and a Trojan-horse type mechanism of toxicity: the case of cobalt oxide on human lung cells. *Part Fibre Toxicol* 11: 14.

NTP response:

Based on a limited literature search, and an assessment of the exposure information (e.g., such as the specificity for cobalt containing implants or levels of cobalt) NTP believes that these studies are not informative for evaluating specific effects of cobalt because of study design (e.g., case reports that have no comparison group), lack of specificity to cobalt (other types of implants or metals in cobalt implants), and inadequate information on the extent of exposure to cobalt, and thus were excluded from the cancer assessment.

A summary of the search methods and list of the studies are available in Appendix A of this document and an overview of the studies is described below. The monograph has been revised to clarify the reasons for not including the implant studies in the evaluation.

NTP identified 30 case reports (many of which were reviewed by Visuri *et al.* 2006) that specifically mentioned that the patient received a joint prosthetic device (e.g., hip, knee, screws) containing cobalt and developed a tumor at the site of the implantation of the prosthesis. The type of tumors were mostly malignant fibrous histiocytoma (12 cases reviewed by Visuri *et al.* 2006, Hughes *et al.* 1987, Lucas *et al.* 2001, Min *et al.* 2008), but also included osteosarcoma (4 reviewed by Visuri *et al.* 2006, Malcom 1984), other types of sarcoma (4 reviewed by Visuri *et al.* 2006, Tayton 1980, Van der List *et al.* 1988), and non-Hodgkin or B-cell lymphoma (Dodion *et al.* 1982, McDonald 1881, Cheuk *et al.* 2005). Case-reports of these types of cancer were also found for non-cobalt containing implants (reviewed by Visuri *et al.* 2006).

NTP identified 16 cohort studies that were primarily record linkage studies conducted in Nordic countries, the United Kingdom, Austria, and the United States, the majority of which did not provide information on the type of implants; most likely these included patients receiving cobalt and non-cobalt containing implants and thus the effects can not be attributed specifically to cobalt. Two cohort studies (Visuri *et al.* 1996, 2010) and a patient-series study (Visuri *et al.* 1996) reported on cancer risk only among patients with McKee-Farrar implants, which contain a cobalt-chromium-molybdenum alloy. However, these studies are also not informative regarding cobalt because of other metals (e.g., chromium, molybdenum, etc.) or chemicals present in the implants, underlying co-morbidities, such as rheumatoid arthritis, which may be a risk factor for the cancer, and because the extent of cobalt exposure is unknown. These studies did not report or stratify by cobalt urine or blood levels, and thus it is likely that the risk (if it exists) of high level exposure would be diluted by the inclusion of patients with low blood or urine cobalt, which may be similar to levels of the comparison group (e.g., the general population) (see Figure 2-2 in the revised monograph document).

Public comments on genotoxicity data

Cobalt Development Institute (CDI) recently published a paper (Kirkland *et al.* 2015),[13] that reported findings from numerous genotoxicity assays on several cobalt compounds, that was not publicly available nor peer reviewed at the time of the release of the draft monograph. Shortly (2 days) before the peer-review meeting, the monograph was accepted for publication and CDI kindly provided a copy to NTP; however, given the extent of the manuscript length of 100 pages,

[13]Kirkland D, Brock T, Haddouk H, Hargeaves V, Lloyd M, McGarry S, Proudlock R, Sarlang S, Sewald K, Sire G, Sokolowski A, Ziemann C. 2015. New investigations into the genotoxicity of cobalt compounds and their impact on overall assessment of genotoxic risk. *Reg Tox and Pharm*. 73 (1): 311-338.

and the very large number (~40) of genotoxicity studies reported in the paper, neither NTP nor the Panel had adequate to time to review the information prior to the peer-review meeting, although it was shared with the Panel members.

NTP Response:

The NTP has decided not to include these genotoxicity studies in the revised monograph because the assessment of these studies was not peer reviewed by the Panel. Due to the large number of studies reported in the Kirkland *et al.* publication and the need for a systematic quality evaluation of the studies (such as for solubility and reporting issues), inclusion of the data in the monograph would require a second peer review which, based on our preliminary review of the data, is unlikely to change the NTP conclusions concerning the carcinogenicity of cobalt, and thus do not justified the additional time, effort, and expense of convening another peer-review panel meeting. An overview of the scope of the paper and NTP's rationale for its decision is provided below.

Kirkland *et al.* (2015) describes the findings for a project consisting of 40[14] genotoxicity studies on 16 different cobalt species (cobalt metal or compounds). One focus of the project was to provide genotoxicity data on "new" compounds (i.e., compounds for which genotoxicity findings are not available in the peer-reviewed literature); 12 of the 16 substances tested were "newly tested" cobalt compounds and the other 4 substances (cobalt metal or metal powder, cobalt sulfate or its heptahydrate, cobalt chloride or its hexahydrate, and cobalt sulfides) were "previously tested" either in different assays or retested in the same assay as reported in the draft monograph.

Another focus was the evaluation of the potential mutagenicity of cobalt; i.e., ~68% (27/40) of all assays were mutagenicity assays, including 20 of the 29 studies of "new" compounds and 7 of 11 of the "previously tested" cobalt species. With respect to the previously tested compounds, only three studies were retested in the same assays as that reported in the monograph. As discussed above, the Panel concluded that there was little evidence that cobalt is mutagenic and thus the negative findings for the majority of the mutagenicity studies reported in Kirkland *et al*. are largely consistent with the findings from the mutagenicity studies included in the draft monograph (see Appendix B of this document for an overview of the studies).

The findings from the other seven in vitro studies reported by Kirkland et al. also appear to be consistent with NTP's conclusions that cobalt causes clastogenic effects. Interpretation of the six in vivo studies (primarily of new compounds) are somewhat more challenging because of unclear findings (see ? in Appendix B table in this document) or the lack of studies evaluating genotoxicity in the target tissue. In an intratracheal-instillation study in rats, De Boeck et al. (2003)[15] found that exposure to cobalt-tungsten carbide caused micronuclei in Type II pneumocytes but not peripheral blood lymphocytes illustrating the potential importance of evaluating target tissue. NTP feels that the evidence from the body of studies reported in

[14]Not including three assays which reported an increase in nuclear anomalies, which can be induced by genotoxic or non-genotoxic mechanisms.

[15]De Boeck M, Hoet P, Lombaert N, Nemery B, Kirsch-Volders M, Lison D. 2003. *In vivo* genotoxicity of hard metal dust: Induction of micronuclei in rat type II epithelial lung cells. *Carcinogenesis* 24(11): 1793-1800.

Kirkland et al. would have little impact on NTP's overall conclusions concerning genotoxicity, the biological plausibility of the mechanisms of carcinogenicity in humans or rationale for grouping cobalt compounds as a class.

Appendix A: Joint Implants

Literature search strategy

The literature search strategy and selection of human cancer studies on joint implants is described below.

PubMed Database was screened using cancer terms for all cancers as well as cancer that has been reported in reviews of case-reports of joint implants combined with terms for joint implants.

Cancer terms

("Neoplasms"[Mesh] OR "Lymphoma"[Mesh]) OR "Sarcoma"[Mesh] OR ("histiocytoma, benign fibrous"[MeSH Terms] OR ("histiocytoma"[All Fields] AND "benign"[All Fields] AND "fibrous"[All Fields]) OR "benign fibrous histiocytoma"[All Fields] OR "histiocytoma"[All Fields] OR "histiocytoma"[MeSH Terms]))

Prosthesis terms

("Hip Prosthesis"[Mesh] OR "Arthroplasty, Replacement, Hip"[Mesh] OR "Arthroplasty, Replacement"[Mesh] OR "Arthroplasty, Replacement, Knee"[Mesh])

Citations (N=1187) were screened using PubMed at the title and abstract level and selected for initial consideration if they were a case-report, case-series, case-control or cohort study reporting on the occurrence of cancer at the site of the joint implantation (case-report or case-series) or cancer risk (cohort studies). Information from 58 case-reports, 1 patient series, and 16 cohort studies regarding the study design, population (including underlying medical conditions), comparison group (if relevant), and type of implant were extracted into an Excel database Technical experts at FDA were consulted regarding type of implants with respect to cobalt content. Of the 58 identified case-reports, 30 specifically mentioned that the implant contains cobalt. Many case-reports (46) were included in a review by Visuri *et al.* 2006, which also had additional information (especially the foreign language studies) or case reports. Two meta-analyses of various types of cancer and hip or knee implant studies were also identified.

Case reports of cobalt implants

1. Aboulafia AJ, Littelton K, Shmookler B, Malawer MM. 1994. Malignant fibrous histiocytoma at the site of hip replacement in association with chronic infection. *Orthop Rev* 23(5): 427-432.

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Appendix B: Genotoxicity and Related Studies

This table summarizes the genotoxicity results reported in Kirkland *et al.* 2015[16] for several cobalt compounds tested in both *in vitro* and *in vivo* assays.

Cobalt compounds	***In vitro* studies**							***In vivo* studies**			
Cobalt Compound[a]	Mutagen Bacteria single strain[b]	Mutagen Bacteria 5 strains[b]	Mutagen Mammalian[c] tk loci	Mutagen Mammalian[c] hprt loci	CA[d]	ROS/ oxidative damage	DNA damage	MN (bone marrow)	CA (bone marrow)	CA (sperm-atogonia)	NA[e]
Acetyl acetonate		neg (5 strains)	pos		±S9 pos			neg			
Borate neodecanoate				neg							
Chloride	TA97 –S9 neg[f]										
Dichloride hexahydrate										neg	
Dihydroxide				neg							
2-Ethyl-hexanoate				neg							
Lithium cobalt dioxide				neg							
Metal	TA98 –S9 neg[f]			–S9 neg							
				+S9 pos							
Metal powder extract				neg							
Monoxide				neg					?[g]		pos
Octoate						pos	pos				
Oxalate				neg							

[16]Kirkland D, Brock T, Haddouk H, Hargeaves V, Lloyd M, McGarry S, Proudlock R, Sarlang S, Sewald K, Sire G, Sokolowski A, Ziemann C. 2015. New investigations into the genotoxicity of cobalt compounds and their impact on overall assessment of genotoxic risk. *Reg Tox and Pharm*. 73 (1): 311-338.

Cobalt compounds	***In vitro* studies**							***In vivo* studies**			
Cobalt Compound[a]	Mutagen Bacteria single strain[b]	Mutagen Bacteria 5 strains[b]	Mutagen Mammalian[c] tk loci	Mutagen Mammalian[c] hprt loci	CA[d]	ROS/ oxidative damage	DNA damage	MN (bone marrow)	CA (bone marrow)	CA (sperm-atogonia)	NA[e]
Oxide hydroxide				neg							
Oxyhydroxide					–S9 pos						
Resinate		neg (5 strains)	neg		+S9 pos (?)			neg			
Sulfate				neg					?[g]		pos
Sulfate heptahydrate	TA100 –S9 neg[f]					pos	pos				
Sulfide				neg							
Tricobalt tetraoxide				neg					neg		pos

CA = chromosomal aberrations; hprt = hypoxanthine guanine phosphoribosyl transferase; MN = micronuclei; NA = nuclear anomalies; neg = negative; pos = positive; tk = thymidine kinase.
[a]Compounds in **bold** had assay results cited in RoC monograph.
[b]Ames test ± S9.
[c]Mouse lymphoma L5178Y cells.
[d]Human lymphocytes or V79 cells.
[e]Nuclear anomalies are not a measure of genotoxicity *per se.*
[f]References cited in RoC monograph reported positive results for these assays.
[g]Reported some positive effects but had toxicity, high mortality and authors call negative.