Cobalt Institute: Response Document for US EPA consultation on IRIS assessment plan and protocol for Co and Co compounds (inhalation, cancer)

Submitted along with the following attachments:

Attachment 1. Stantec ChemRisk Cobalt Comments IRIS

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: ci@cobaltinstitute.org
www.cobaltinstitute.org

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18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: ci@cobaltinstitute.org
www.cobaltinstitute.org

Executive Summary

Cobalt and cobalt compounds were nominated by the EPA's Office of Air and Radiation (OAR), in June 2022 for a cancer assessment under the US EPA IRIS Program due to potential carcinogenic risks arising from emissions to air of cobalt compounds during industrial processes. The Cobalt Institute (CI) is submitting comments in December 2022, relating to the consultation on the IRIS Assessment Plan (IAP) and protocol for Co and Co compounds.

The CI recently published a series of papers containing data on the cobalt mode-of-action (MOA) relating to longer-term inhalation toxicity and carcinogenicity of cobalt and cobalt compounds. In these publications, the CI has performed a tiered, **mode-of-action based read-across approach**, detailing the distinct differences in the release of the Co ion in artificial lung fluids, upregulation of in vitro biomarkers for hypoxia, inflammation, oxidative stress and acute toxicity/persistent inflammatory responses and sub-acute toxicity, between the 'reactive Co substances' (represented by Co metal and Co sulfate) and the 'poorly reactive / poorly soluble' Co substances (represented by tricobalt tetraoxide). Based on this and other extensive datasets, **Co metal is not considered as part of the poorly soluble group of substances**. In contrast, tricobalt tetraoxide is poorly soluble in all lung fluids and high quality, mode-of-action data indicates a lower potency and potential alternative cancer MOA for poorly soluble cobalt substances. The existence of different groups of cobalt substances, and the grouping of Co metal with the reactive substances, is supported by the recent IARC review of Co and Co compounds. In addition, mutagenic responses (i.e. direct genotoxicity) have not been detected in guideline-compliant studies with any cobalt substance, including the substances that were positive for cancer by inhalation exposure in rodent studies.

The CI proposes that the EPA uses a **weight-of-evidence approach** to assess the dose-response for reactive, bioavailable cobalt substances taking into account MOA, animal and epidemiological (workplace) data. **A multi-year testing program** has been provisionally approved and will inform on the MOA, dose-response and tissue deposition of poorly soluble/ poorly reactive Co substances and in vivo site of contact genotoxicity and inflammatory responses induced by reactive Co substances.

Combined, all evidence on the carcinogenicity of cobalt can be reconciled into **one mode-of-action paradigm and into one continuous exposure response with a good estimation of a threshold in the dose response curve**. The essential biological role of cobalt and its extensive mechanistic, genotoxicity and toxicity data fit the overall profile of a carcinogen with a threshold mode-of-action and this is consistent with the available epidemiological data. In contrast, current linear approaches based solely on animal data yield a predicted excess

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: ci@cobaltinstitute.org
www.cobaltinstitute.org

cancer risk that cannot be reconciled with the **lack of excess cancer cases observed in the epidemiology studies**.

In both NTP cancer bioassays (cobalt sulfate and cobalt metal powder), adrenal pheochromocytoma were observed. These tumors are a well-known secondary response to respiratory distress of any origin (not just lung cancer) and should not be interpreted as independent cobalt-related tumors. Further systemic tumors were only seen in one sex of rats (not in mice) in the cobalt metal powder study. The rat strain and colony used in this inhalation study does not have a historical control database against which these tumors can be compared, making the interpretation of these findings extremely difficult. Further, there was no exposure-response in any of these findings**. The conclusion of independence of these tumors appears implausible**.

The CI promotes the responsible and sustainable production and use of cobalt in all its forms and applications. The Institute aims to protect and grow the market for cobalt and compounds by promoting a proportionate regulatory environment. We welcome any additional requests or clarifications from the US EPA regarding the current submission and would appreciate the opportunity for cooperation and communication to lead to the appropriate risk management measures and limit values applied to cobalt and cobalt substances.

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
www.cobaltinstitute.org

1. Cobalt and Cobalt Substances

Summary:

- At least two groups of Co substances exist as supported by existing data and a new testing strategy that has compiled evidence on mode-of-action and toxicity of different cobalt substances.
- Co metal is highly soluble in biologically relevant fluids and belongs in the 'reactive' / bioavailable Co substance group.
- Tricobalt tetraoxide is a poorly soluble (in all biologically relevant fluids) and belongs to the poorly soluble / poorly reactive group.
- Mutagenicity has been excluded as a MOA leading to Co-induced carcinogenicity.
- The IARC has concluded on carcinogenicity classifications for the reactive cobalt substances and lack of classification for the poorly reactive Co substances.
- The CI will generate additional MOA and toxicokinetic data to inform on the poorly soluble substances (i.e. sub-chronic data).
- The CI will generate additional genotoxicity data to inform on the reactive Co substances (i.e. quantitative thresholds for genotoxicity and inflammation).

Overview of CI grouping and cobalt-induced carcinogenicity

The CI has developed a detailed read-across and grouping approach for longer-term inhalation toxicity and carcinogenicity. Both Co metal and certain Co substances share similar properties relating to predicted carcinogenic effects and reduction in lung function ('workplace asthma'). With moderate solubility in neutral aqueous fluids, and after undergoing significant corrosion processes with high release of Co ion in acidic media (i.e. lysosomal and gastric fluid), cobalt metal powders can release a sufficient amount of Co^{2+} ion (toxic unit of interest) to activate key events in the mode-of-action (MOA) for cobalt carcinogenicity. The 5 soluble cobalt salts[1] are highly soluble in both neutral and acidic fluids and release sufficient amount of Co^{2+} to activate the same key events in the MOA as Co metal.

In addition, using the CI read-across approach (please see subsequent sections of this document), *certain* cobalt substances were observed to release similar amounts of Co^{2+} in vitro (i.e. bioaccessibility testing) and activate key in vitro and in vivo elements in the mode-of-action pathway shared with cobalt metal and the 5 cobalt salts. The MOA for reactive cobalt substances consists of cytotoxicity, oxidative stress, hypoxia and inflammatory effects, that occur in combination and are required before observing the ultimate adverse outcome. This has been outlined in the CI inhalation read-across publications (see subsequent sections of this document). These additional substances were self-classified by the cobalt industry as

[1] Cobalt dichloride, cobalt sulfate, cobalt dinitrate, cobalt diacetate, cobalt carbonate (and hydrated forms).

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: ci@cobaltinstitute.org
www.cobaltinstitute.org

category 1B carcinogens under EU CLP[2] and known as 'reactive' cobalt substances (e.g. Co monoxide, Co dihydroxide).

In contrast, there are cobalt substances (e.g. tricobalt tetraoxide, cobalt sulphide, cobalt lithium dioxide, cobalt hydroxide oxide) that *are not* predicted to release sufficient amounts of Co^{2+} ion in aqueous (neutral and acidic) media to activate key events in the cobalt MOA (i.e. which would lead to the ultimate adverse outcome of carcinogenicity) and instead are likely to activate alternative pathways associated with 'poorly soluble particles'.

Data generation and application of the inhalation read-across approach to cobalt substances with organic ligands (i.e. cobalt 'carboxylates') is still on-going. Results are expected in the next few years.

The CI produced a series of publications that differentiated between the 'reactive' and 'poorly reactive / poorly soluble' cobalt substances and highlighted data available showing the differences in response between the two groups in a detailed mode-of-action paradigm. This MOA paradigm consists of release of the cobalt ion in artificial biological lung fluids (tier 1), in vitro biomarkers (tiers 2a and 2b), acute inflammatory responses and 'persistent' inflammatory markers (tier 3) and a 28-day repeated-dose inhalation toxicity study (tier 4), with a 90-day recovery period (Verougstraete et al., 2022; Derr et al., 2022; van der Brule et al., 2022; Viegas et al., 2022; Burzlaff et al., 2022 and Danzeisen et al., 2022). It was observed that the poorly soluble/poorly reactive cobalt substances do not show the same pattern in activating reactive inflammatory markers, hypoxia and oxidative stress – and importantly, not at the same magnitude in dose / concentration level as the reactive cobalt substances.

Please see Annex 1 of this document for relevant details (phys-chem properties) related to cobalt and cobalt substances (both reactive and poorly reactive / poorly soluble) considered within the CI inhalation read-across approach.

Mutagenicity

Mutagenic responses (i.e. direct genotoxicity) have not been detected in guideline-compliant studies with any cobalt substance (including the substances that were positive for cancer by inhalation exposure in rodent studies). The relevant events in the reactive Co MOA is depicted in the mode-of-action diagram (see Figure 1). The CI has excluded direct in vivo genotoxicity as a major mode-of-action of cobalt-related cancer based on (1) data generated under EU REACH for the endpoint mutagenicity, including ToxTracker data[3], (2) a recent OECD CoCAM

[2] Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP Regulation)

[3] The ToxTracker assay (Toxys) is a stem cell-based reporter assay that provides mechanistic insights into the genotoxic properties of chemicals, contributing to a mechanism-based, animal-free, cancer hazard and risk assessment of chemicals. It is currently undergoing review to become an OECD guideline testing method.

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
www.cobaltinstitute.org

conclusion on four soluble Co salts[4], and (3) an extensive Co-related genotoxicity database publication by D. Kirkland (2015)[5]. Mutagenicity is therefore not considered a relevant driver of cobalt-related carcinogenicity and is not considered as a possible early marker of cancer in this cobalt–specific read across paradigm. In addition, to quantitatively support this lack of mutagenicity and indirect genotoxicity events, the CI has proposed two in vivo genotoxicity studies (detailed in the subsequent sub-section), and have received provisional approval from the European Chemicals Agency (ECHA) to proceed with testing.

Cobalt metal belongs in the reactive group

Cobalt metal powder is poorly soluble in water, however it is moderately to highly soluble in biological fluids, such as interstitial, alveolar or lysosomal artificial lung fluids. Data on the bioelution of several Co compounds in lung fluid has led to the grouping of Co metal powder with the soluble cobalt salts (Co sulfate, Co chloride, Co nitrate and Co acetate) in one group of Co compounds classified as inhalation carcinogens in the EU (Carc. 1B). This group of compounds is characterized by the induction of an inflammatory response and hypoxia in the lung following inhalation exposure. The data in the recent publication series outline the similarities of Co metal with other reactive Co substances. The similarity in effects caused by this group of substances has led to the conclusion that the toxicity of Co compounds is related to the Co ion, and that the magnitude of effect is related to the Co ion dose-to-target. This also inherently assumes that dose-to-target is critical for the magnitude of effect, and not differences in the potency between Co substances. This assumption is confirmed by the evaluation of the dose-response of Co exposure (from Co sulfate and Co metal powder) across all exposure concentrations in both NTP studies.

It is important to note that there are substances with negligible solubility in biological fluids (e.g., tricobalt tetraoxide and cobalt sulfide). Bioelution data are provided in the table in the appendix, indicating that these ‘biologically insoluble’ substances should not be grouped with Co metal powder for the endpoint inhalation toxicity.

Recent IARC conclusions for Co and Co compounds

In March 2022, an in-depth scientific evaluation of cobalt and cobalt compounds was performed by IARC. Cobalt metal and soluble cobalt (II) salts were classified as ‘probably carcinogenic to humans’ (Group 2A); cobalt (II) oxide was classified as ‘possibly carcinogenic

[4] OECD (2014). "SIDS Initial Assessment Profile Soluble Co salts CoCAM 6. http://webnet.oecd.org/hpv/ui/handler.axd?id=e5e60085-1f3f-4df5-92f6-8f32c26c3082;

[5] Kirkland, D., T. Brock, H. Haddouk, V. Hargeaves, M. Lloyd, S. M. Garry, R. Proudlock, S. Sarlang, K. Sewald, G. Sire, A. Sokolowski and C. Ziemann (2015). "New investigations into the genotoxicity of cobalt compounds and their impact on overall assessment of genotoxic risk." Regul Toxicol Pharmacol **73**(1): 311–338.

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
www.cobaltinstitute.org

to humans' (Group 2B) and cobalt (II, III) oxide, cobalt sulfide and other cobalt (II) compounds were evaluated as 'not classifiable as to its carcinogenicity in humans' (Group 3).

The CI states that the IARC conclusions as further justification to separate tricobalt tetraoxide, Co sulfide and other Co (II) substances from Group 2A or 2B Co substances (e.g. Co metal, soluble Co salts, Co monoxide and other 'reactive' Co substances), and further support to place Co metal with the reactive Co substances.

Multi-year testing program to inform on MOA and genotoxicity

Sub-chronic toxicity study and in vivo 'site of contact' genotoxicity studies

The CI has recently (i.e. October 31st, 2022) received provisional approval from the European Chemicals Agency to conduct a 90-day sub-chronic toxicity study with tricobalt tetraoxide. The data generated in the 90-day study would inform on the IRIS assessment for the poorly sluble / poorly reactive group of substances, specifically:

- Evaluation of dose-response
- Co levels in blood and tissue
- Markers related to reactive Co substances (i.e. 8-OH-dG, HIF-1alpha upregulation, inflammation, etc.)
- Lung burden measurements and comparison to poorly soluble particle MOA

The CI has also recently (i.e. October 31st, 2022) received provisional approval from ECHA to conduct two in vivo 'site of contact' genotoxicity studies (in vivo transgenic rodent assay and in vivo COMET assay), with cobalt sulfate. The data generated in these two studies would inform the IRIS assessment of reactive Co substances, specifically:

- Quantitative assessment of direct and indirect genotoxicity at site of contact and in other relevant tissues (i.e. bone marrow, pancreas, kidney, etc.)
- Co levels in blood and tissue
- Assessment of inflammatory markers
- Assessing a threshold for indirect genotoxicity and inflammation

The CI also would like to alert the EPA that provisional approval to proceed with testing has been given for a combined chronic / carcinogenicity study with Co dichloride via the oral route.

We estimate the data from the studies detailed above will be available from 2025 onwards.

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
www.cobaltinstitute.org

2. Cobalt Institute MOA based tiered read-across approach

Summary

- The CI MOA tiered read-across and grouping approach was published in a series of papers in Regulatory Toxicology and Pharmacology in 2022.
- The tiered testing approach uses MOA data to predict longer-term toxicity of Co substances.
- High quality in vitro and in vivo data have demonstrated the cobalt-associated mode-of-action for carcinogenicity includes oxidative stress, cytotoxicity, hypoxia and a sustained inflammatory response – all of which are threshold events.
- The tiered approach has provided further data to prove the existence of at least two groups of Co substances.

Cobalt sulfate and cobalt metal were observed to cause lung tumours following inhalation by rats and mice in two NTP carcinogenicity studies[6]. Based on these data, both substances met the criteria for classification under the EU CLP Regulation as carcinogenic Category 1B[7] (presumed to have carcinogenic potential for humans). The CI therefore evaluated whether further Co compounds meet the criteria for classification under the same endpoint. However, it is neither desirable (for animal welfare reasons) nor possible to conduct lifetime inhalation carcinogenicity studies on every Co-containing substance for the purpose of determining inhalation carcinogenicity hazard. The EU CLP Regulation foresees the 'read-across' of the carcinogenicity classification to a structurally similar substance; such an approach "must always be based on a robust and transparent argument" and must take into account other important factors such as physico-chemical, toxicokinetic and any other available relevant information.

Using a robust scientific approach, Cobalt Institute developed a mode-of-action tiered testing approach for cobalt and cobalt substances that are registered by CI under EU REACH, to employ a read-across and grouping approach to address longer term inhalation toxicity and carcinogenicity. The full data sets relating to the tiered read-across approach are detailed in the EU REACH registration dossiers and the concise data are contained within a series of papers in the public domain submitted in 2021 (Verougstraete et al., 2022; Derr et al., 2022;

[6] Bucher, J. R. (1998). Toxicology and carcinogenesis studies of cobalt sulfate heptahydrate (CAS NO. 10026-24-1) in F344/N rats and B6C3F1 mice (inhalation studies) National Toxicology Program. **NTP TR 471**. Behl, M. and M. J. Hooth (2014). Toxicology studies of cobalt metal (CAS NO. 7440-48-4) in F344/N rats and B6C3F1/N mice and toxicology and carcinogenesis studies of cobalt metal in F344/NTac rats and B6C3F1/N mice (inhalation studies), National Toxicology Program. **NTP TR 581.**

[7] Cobalt metal has a classification for carcinogenicity via 'all routes of exposure' due to the lack of conclusive evidence of lack of carcinogenicity via other routes of exposure (CLP regulation). The CI and Nickel Institute have submitted a testing proposal for oral carcinogenicity to fill this knowledge gap.

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: ci@cobaltinstitute.org
www.cobaltinstitute.org

van der Brule et al., 2022; Viegas et al., 2022; Burzlaff et al., 2022 and Danzeisen et al., 2022[8]).

In summary, based on high quality in vitro and in vivo data, the cobalt-associated mode-of-action for carcinogenicity includes oxidative stress, cytotoxicity, hypoxia and a sustained inflammatory response – all of which are threshold events. See Figure 1, below for the pictorial representation. The MOA for the poorly soluble/poorly reactive group of substances is hypothesised in Figure 2, below.

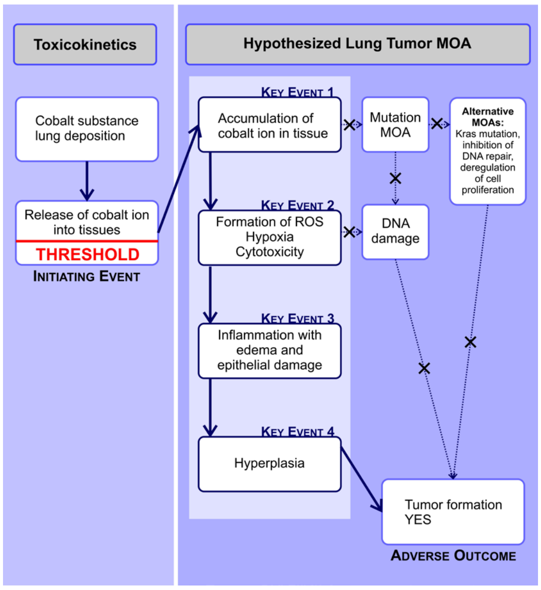

Figure 1. Proposed MOA for reactive cobalt substances

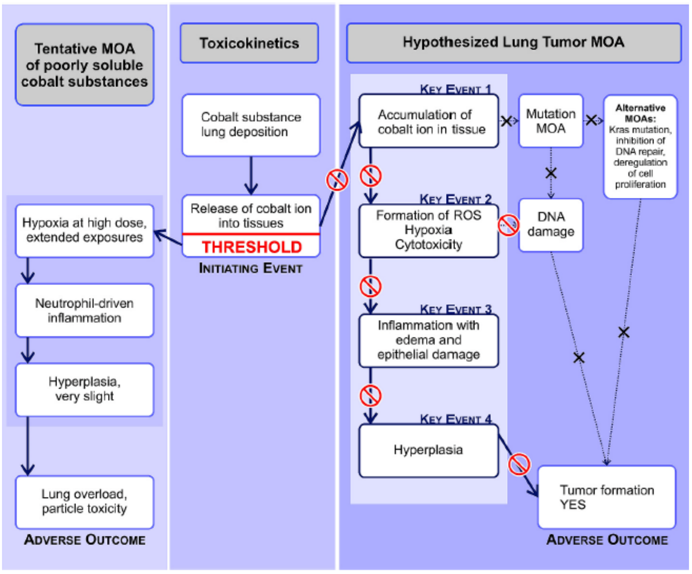

Figure 2. Hypothesised MOA of poorly soluble cobalt substances

[8] Due to upload limitations, links to the open access papers will be provided in this document.

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
www.cobaltinstitute.org

The extent to which a cobalt-containing substance can induce these events can be measured, and these 'markers' are used in a weight of evidence approach to group cobalt-containing substances based on their predicted potential to induce such chronic inhalation effects (i.e. carcinogenicity = reactive cobalt substances; lack of carcinogenicity = poorly reactive cobalt substances). For example, predicting longer-term inhalation effects on the basis of 'middle' or 'lower' tiers such as the measurement of acute in vivo responses and in vitro biomarkers, respectively (see Figure 3 for a pictorial representation).

Several tiers of testing have been defined to complete the read-across evidence base (Figure 3). They range from the lower (less weighted in terms of relevance) tiers of in vitro testing, up to mid-level tiers of in vivo acute and repeated-dose inhalation testing and conclude with the highest tier, a two-year rodent carcinogenicity study. The lower tiers are aimed at making distinctions between the various cobalt compounds based on common toxicological drivers (e.g. hypoxia, cytotoxicity, oxidative stress), whereas the higher tier testing is aimed at "proving the concept" of the existence of different groups of compounds regarding chronic inhalation toxicity endpoints (e.g. chronic pulmonary inflammation, metaplasia).

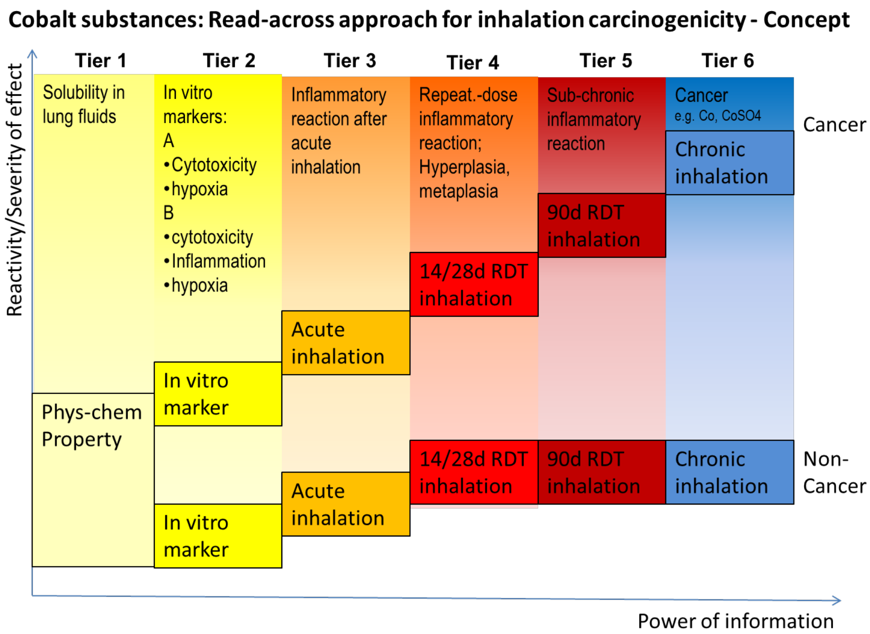

Figure 3. Cobalt inhalation read-across and grouping approach: Tiered testing strategy

In Figure 3, each tier is ranked from left to right in terms of increasing power of information, with the cancer tier ranked as the highest. Currently, Co substances are separated out into two distinct groups based on reactivity and severity of local lung effects:

- Substances that are 'poorly reactive' across the different tiers are placed in the 'non-cancer/lack of chronic toxicity' group

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
www.cobaltinstitute.org

- The 'poorly reactive' group is awaiting data from a 90-day repeated-dose inhalation study to further define the toxicity associated with these substances
- Substances that are reactive across the tiers are placed in a chronic inhalation toxicity (cancer) grouping with cobalt sulfate and cobalt metal.
- Substances that show a 'disparity' (i.e. tiers 1, 2 are not aligned with tier 3 outcomes) will be tested in further, higher tiered tests

As a direct result of the tiered read-across approach and data generated, Co monoxide and Co dihydroxide were self-classified as **Carc. 1B; H350i** by the Cobalt Institute in January 2017 and January 2018, respectively.

The CI inhalation read-across approach was validated with cobalt substances that had existing in vitro and in vivo data to inform on MOA. Testing is still on-going for Co substances in which toxicity data from other routes of exposure (e.g. oral route) have demonstrated a difference in MOA to cobalt substances with inorganic ligands. Specifically, the CI read-across approach is still being applied for cobalt substances with organic ligands (i.e. cobalt carboxylates) in which preliminary data suggest a disparity within the tiers, which warrants further, higher tier testing.

Based on the inhalation read-across and grouping approach, the Cobalt Institute has derived health-based limit values for: cobalt metal, cobalt sulfate, cobalt dichloride, cobalt diacetate, cobalt dinitrate, cobalt carbonate, cobalt monoxide and cobalt dihydroxide. These substances consist of the 'reactive' group of cobalt substances, hypothesised to lead to a decrease in lung function (based on epidemiology studies) and carcinogenicity at higher exposure levels (based on rodent carcinogenicity studies).

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
www.cobaltinstitute.org

3. Dose-response for cobalt substances – Using a weight-of-evidence approach

Summary

- The genotoxicity database for Co and Co substances excludes direct genotoxicity as a MOA for cobalt-induced carcinogenicity.
- A recent, high quality, large epidemiology study conducted in the hard metal industry did not find an increased risk of cobalt-induced cancer.
- Using a weight-of-evidence approach for the reactive cobalt substances, high quality human epidemiology data (negative for cancer) can be used to inform on carcinogenic risk at the low-end of the dose-response curve, when using the NTP rodent carcinogenicity data (positive for cancer) for quantitative analysis.
- Human epidemiology data do not support a linear extrapolation at low doses, nor do they support a high potency for cancer.
- The CI has scientific concerns regarding the layers of conservative assumptions that are required within a linear extrapolation at low doses in the inhalation unit risk estimate for carcinogenicity for cobalt and cobalt substances
- Human data show the first adverse effect in the respiratory tract is linked to a reduction in lung function 'workplace asthma' – this has a quantitative threshold.

It is known that the relevant hazards and health effects following inhalation exposure to cobalt substances are related to the respiratory tract. These manifest as an inflammatory response which, if sustained, could lead to tumours of the respiratory tract. The latter hazard has only been observed in animals. Both effects are postulated to have thresholds. There is adequate data to inform on the dose-response for carcinogenicity in rodents induced by the reactive Co substances. There is also adequate data to inform on tissue deposition after inhalation of reactive cobalt substances, as detailed in the 90-day NTP dose-range finding study with cobalt metal powder.

Longer-term data to inform on the MOA, dose-response and tissue deposition of the poorly reactive Co substances will be generated in the upcoming years. This data is needed in order to perform an evidence-based derivation of the dose-response for the poorly reactive substances.

Attachment 1 to this response document (i.e. Stantec ChemRisk – Comments on IRIS Cobalt Assessment Plan) details the strengths and weaknesses of prior reviews of the carcinogenicity and dose response of cobalt (i.e OEHHA, TCEQ and ECHA), along with a summary of current

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: ci@cobaltinstitute.org
www.cobaltinstitute.org

critical reviews of cancer dose-response methods and an analysis of cobalt epidemiological data with regards to predicting an increase in cancer risk and cancer potency.

Regarding the derivation of exposure-response relationships related to the reactive cobalt substances, we have identified and detailed below, (1) epidemiology data relating to a decrease in lung function and (2) epidemiology data detailing a lack of carcinogenicity observed in the workplace, used in a weight of evidence approach alongside the NTP rodent carcinogenicity studies. As cobalt-induced carcinogenicity has not been observed in the workplace, we have instead provided data on a respiratory effect observed in cobalt workplaces, with a quantitative threshold. High quality, recent and robust epidemiology studies were conducted in both cobalt-only workplaces and in the hard metal industry, in which no increase in cancer risk was observed after cobalt exposure. The use of hard metal epidemiology data, when relating to lack of cancer observed in the workplace, is still relevant to cobalt-only workplaces, as cobalt in combination with tungsten carbide is more potent / reactive than cobalt alone. Put simply, if no increase in cancer risk was seen after exposure to cobalt in hard metal, then no increase in risk is expected with cobalt alone at the exposure levels experienced in this study.

Inhalable fraction – Decrease in lung function

Data collection identified high quality epidemiology studies to inform on a decrease in lung function ('workplace asthma'). Relevant details from these studies have been summarised below.

Based on the cohort exposed to cobalt metal, oxides and salts in the Finnish cobalt plant (Linna et al. 2003; Roto 1980; Sauni et al. 2010), exposure to cobalt at levels of 6 - 1,000 µg Co/m³ was reported to induce occupational asthma in those workplaces where irritant gases like sulphur dioxide, hydrogen sulphide or ammonia were also present in addition to cobalt, or the workers had smoking habits. In the absence of irritant gases, cobalt exposure did not lead to occupational asthma at a median cobalt exposure level 120 µg/m³ (min to max 20 – 300 µg/m³). It should also be noted that the authors reported that none of the workers with occupational asthma showed a positive skin prick-test result and most showed late or dual reactions in a bronchial challenge test, indicative of respiratory irritation rather than a sensitisation response.

In addition, occupational exposure to cobalt compounds (2 - 7700 µg/m³; geometric mean TWA 125 µg/m³ and in 25% > 500 µg/m³) in a Belgian cobalt refinery was reported to be associated with a higher incidence of dyspnoea and wheezing as well as allergic contact-

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
www.cobaltinstitute.org

dermatitis, manifesting as eczema and erythema compared to a non-exposed control population (Swennen et al. 1993; Verougstraete et al. 2004). In this study no difference in respiratory volumes, ventilatory performance, carbon monoxide diffusion capacity or lung abnormalities in chest radiography were observed between the cobalt exposed and the control population. The reduction in FEV1[9] in the cobalt exposed worker population, as documented by urinary cobalt levels, was only associated with smoking habits.

Overall, there may be a risk for obstructive lung disease in workers with smoking habits, if exposed above mean and median levels of 120 and 125 µg Co/m^3, respectively. There was no sign of restrictive lung disease in any of the cohorts, based on, e.g., measurements of FEV1/VC[10], carbon monoxide diffusion capacity or chest radiography.

A repeated dose inhalation hazard (non-cancer) of respiratory irritation, measured as a reduction in FEV1[11] has been identified. The FEV1 measurement is an indication of a functional impairment that can be caused by inflammation, irritation, or “workplace asthma” that has been reported for cobalt and cobalt compounds. Lung impairment has been studied by the investigation of additional endpoints, such as lung radiographs and several other physiological parameters (Swennen et al. 1993). The data collection and review identified a key study, where a relatively large cohort from a cobalt plant in Finland (cobalt metallurgical plant in Kokkola that started operations in 1966) was studied by several authors through three decades. The key study used in the calculation of the non-cancer limit value should bethe recent publication from this cohort related to occupational asthma (Sauni et al. 2010).

Studies on occupational asthma from the diamond polishing industry have been excluded due to relevance. We detail the following deficiencies associated with these studies:

- Correlation between exposure to Co and reduction in FEV only observed at group level;
- Not possible to conclude cause-and-effect relationship between the average intensity of Co exposure and ventilatory function: Individuals with elevated Co exposure (by urine measurement) were not identical to those displaying decreased lung function (FEV1);
- Co-exposure to diamond dust as well as other abrasion products from the polishing disks;
- No information on particle size distribution in the workplaces.

[9] FEV1 = forced expiratory volume during 1 second
[10] VC = vital capacity
[11] FEV1 = forced expiratory volume during 1 second

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
www.cobaltinstitute.org

Respirable fraction – Cancer

Epidemiology data

The Marsh study (Marsh et al. 2017b) is a pooled analysis of data from an international study to evaluate total and cause-specific mortality of current and former hard metal production workers with emphasis on lung cancer. The workers' exposure to Co, tungsten (W) and/or Ni was estimated by the construction of job-exposure matrices (JEMs) over the time period 1952 to 2014; exposure estimates were calculated from available company industrial hygiene measurements. An external comparison with the corresponding national and local populations was performed, as well as an internal comparison with adjustment for potential co-exposures and confounding factors, such as smoking. The pooled cohort consisted of 32,354 workers, resulting in 798,330 person-years of observation or an average of 24.7 person-years per worker, from three companies and 17 manufacturing sites in five countries. Standardised mortality ratios were computed and exposure-response via relative risk regression analysis was evaluated.

Among long-term workers, overall deficits or slight excesses in deaths for total mortality, all cancers, and lung cancer were found. There was no evidence of any exposure-response relationship for lung cancer. The study authors therefore concluded that they found no evidence that duration, average intensity, or cumulative exposure to tungsten, cobalt, or nickel, at levels experienced by the workers examined, increases lung cancer mortality risks. There was also no evidence that work in these facilities increased mortality risks from any other causes of death or from any other cancer.

The authors state that one particular challenge in their study was to properly account for the marked elevations in total and certain cause-specific mortality risks observed in the Swedish and German cohorts (Morfeld et al. 2017; Westberg et al. 2017a), compared with mostly deficits in deaths observed in the Austrian, US, and UK cohort (Marsh et al. 2017a; McElvenny et al. 2017; Wallner et al. 2017). Sweden comprised 45.7% of the total pooled cohort of 32,354 workers and 69.5% of the 7187 total observed deaths; Germany represented 21.1% of the pooled cohort and 10.4% of the total deaths.

Another challenging aspect of this study is its inclusion of a large number of short-term workers in the overall cohort (30.4% of the pooled cohort worked less than 1 year and 55% worked less than 5 years), and a disproportionate percentage of short-term workers in the Swedish and German cohorts (40.7 and 22.2%, respectively). The typically unhealthy behavioural and lifestyle characteristics associated with short-term workers often lead to less favourable mortality outcomes compared with longer-term workers. The study authors accounted for these cohort features by stratifying the pooled mortality analyses by worker "type 1" (either <1

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
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or +1 years of employment), by adjusting exposure-response models for the factors worker "type 1" and/or country, and by performing certain analyses with and without the inclusion of the Swedish cohort data.

A potential weakness of the study is the possible influence of the ''healthy worker effect'', e.g. through the positive health effects of continued employment, and better health care access. Although all risk estimates were standardised for age, the study populations in most countries were young, contributing a relatively small percentage of total deaths before the end of the observation period (e.g., in Austrian, German, UK, and US cohorts, only 9.0, 11.0, 11.9, and 14.9%, respectively, of workers had died by the end of follow-up). Consequently, many members did not reach the older age groups for which mortality rates for many "cause of death" categories begin to increase significantly in the general population.

On the other hand, this pooled analysis of US and EU hard metal workers has many methodological strengths, especially when compared with other epidemiological studies from the hard metal or cobalt industry. It included 32,354 workers from three companies and 17 manufacturing sites in five countries (eight US, three German, three Swedish, two UK, and one Austrian), each independently conducted under the direction of country-specific occupational epidemiology experts. The pooled analysis included a comprehensive, quantitative study member-level assessment of exposure to tungsten, cobalt, and nickel that enabled robust RR regression modelling of exposure-response with control for potential confounding factors.

Overall, an excellent mortality follow-up was attained and cause of death ascertainment rates that covered a long 63-year (1952–2014) observation period were reached. The pooled cohort included a total of 459 lung cancer deaths, of these, 287 were long-term (1+ yr) workers, that yielded excellent statistical power to detect excesses in deaths overall and in most of the subcategories examined.

Confidence in epidemiological studies is often measured by a study's ability to detect <u>known</u> carcinogens. In this context it is important to point out that tungsten is not carcinogenic[12]. Also Ni metal is not carcinogenic in humans, nor in animals[13]. Negative inhalation exposure carcinogenicity data in rodents exist for Ni metal (Oller et al. 2008) and it is classified as a category 2 carcinogen by read-across from Ni compounds only[14]. The lack of an increased

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[12] W (tungsten) does not carry any CMR classification under CLP or IARC.
[13] Only Ni_3S_2 and NiO are inhalation carcinogens in rodents and humans, and $NiSO_4$-hexahydrate is an inhalation carcinogen in humans only. Ni metal was classified as Carc 2 (EU CLP) and Cat 2 B (IARC) by read-across from these compounds, despite negative carcinogenicity data (negative rodent cancer bioassay by inhalation, Oller et al 2008, and no evidence for carcinogenicity in humans).
[14] NiPERA fact sheet on carcinogenicity: https://nickelinstitute.org/science/human-health-fact-sheets/fact-sheet-2-nickel-nickel-compounds-carcinogenicity/

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
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cancer risk associated with those exposures in the Marsh et al. pooled cohort does therefore not decrease the confidence in this study.

In terms of its statistical power and in terms of exposure description, the pooled cohort study by Marsh et al. (Marsh et al. 2017b) is of excellent quality. The lack of finding of “hard metal disease” (lung fibrosis) is probably related to the relatively low exposures experienced in this cohort, with the average intensity of exposure (AIE) of a ratio mean[15] of 13 µg Co/m^3 and a maximum AIE of 300 µg Co/m^3. The lack of finding of lung cancer related to Co exposure is an indication that either Co is a carcinogen only in rodents and not in humans, or that the Co exposures experienced in this cohort are below the threshold for the development of cancer. It is not possible to calculate a dose-response for cancer in relation to Co exposure from this study. A plausible conclusion from the data is, that the threshold for cancer in humans is above the exposures experienced in this cohort.

The aim of the study by Sauni et al. (Sauni et al. 2017) was to assess the risk of cancer among workers employed in a Finnish cobalt plant since the beginning of production in 1968. The study cohort consisted of all males employed by the Finnish cobalt plant for at least a year during 1968–2004. The follow-up for cancer was performed by studying the files of the Finnish Cancer Registry, using personal identity codes as a key and allowing complete follow-up of each study participant. The cohort was divided into sub-cohorts by exposure levels. Most participants (n = 380; 11,255 person-years) were in the “high” exposure group, with a mean exposure (TWA) of above 60 µg Co/m^3 throughout the operation of the plant. The “moderate” and “low” exposure groups contained n = 159 (2823 person-years) and n = 364 (9967 person-years), respectively, corresponding to mean TWAs of between 20 – 30 µg Co/m^3 (moderate) and below 20 µg Co/m^3 (low). A fourth group (n = 110, 2174 person-years) undertaking maintenance tasks in the various departments of the plant was assigned a “variable exposure with peak exposures” without numerical TWA values.

Standardised incidence ratios (SIRs) and 95% confidence intervals (95% CIs) were calculated as ratios of the observed numbers of cancer cases and the numbers expected on the basis of incidence rates in the population of the same region. The follow-up cohort consisted of 995 men with 26,083 person-years. During the follow-up period, 92 cases of cancer were diagnosed (SIR 1.00, 95% CI 0.81–1.22), six of which were lung cancer cases (SIR 0.50; 95% CI 0.18–1.08). The only cancer type with increased incidence was tongue cancer (three cases, SIR 7.39; 95% CI 1.52–21.6).

[15] Ratio mean computed as: sum (CUM)/sum (DUR) across all workers with known work history.

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There was no dose-response effect across the different exposure levels and the incidence of any cancer type. The results suggest that occupational exposure to cobalt is not associated with an increased overall cancer risk or lung cancer risk among cobalt workers. However, because of the small number of cancer cases the results must be interpreted with caution.

A potential weakness of the study is its small cohort size with only 92 cancer cases, six of them cancer of the lung. Also, the high SIR for tongue cancer remains unexplained, although the authors state that the three cases were all smokers and their alcohol consumption was not known (both exposures are the most significant risk factors for tongue cancer, whereas this type of cancer has not been associated with Co exposure in any previous study). Because the small number of tongue cases here, the excess may be explained by chance alone, according to the authors. Further, exposures are reported as AIEs (corresponding to the TWA of an OEL), rather than individual cumulative exposures (as was done in the Marsh et al pooled cohort).

Despite these aspects, this study also has several compelling strengths: the precise knowledge of the exposure concentrations (TWAs) due to access to industrial hygiene measurements, including urinary cobalt measurements to assess systemic exposures. This represents a significant improvement over the previous studies from the French cohort (Moulin et al. 1993; Mur et al. 1987), where the exposure levels were unknown. Mur et al. (Mur et al. 1987) reported an increased SMR for lung cancer among cobalt workers, although a follow-up study of the same cohort with an extended observation period could not verify those results (Moulin et al, 1998). In the French cohort, the compounds workers were exposed to were comparable to those in the Sauni cohort. The Sauni et al. (2017) cohort study adds to the negation of the conclusions by Mur et al. (1987) and corroborates the study by Moulin et al. (1998), where no association between Co exposure and cancer in humans was observed.

The healthy worker effect was a concern flagged by the authors of the pooled hard metal cohort (Marsh et al. 2017b). Sauni et al. (Sauni et al. 2017) conclude that the healthy worker effect did not play a marked role in their study, as in the Finnish cobalt plant there was no selection of workers because of possible cancer risk (no pre-employment health checks to exclude individuals that could have elevated cancer risk). Also, the workers were followed and included in the study even after the end of employment. Thus, if they had to leave work because of health reasons, they were still included in the Finnish cohort. The complete follow-up of cause of death of all study participants based on the Finnish cancer registry is in itself a great strength of the study, and it also minimises any confounding by health worker effect.

Based on several considerations, e.g. study design, length of observational period covered, existing co-exposures, size of cohort, detail in reporting and knowledge of Co exposure

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
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levels etc, the Finnish cohort described in Sauni et al. (Sauni et al. 2017) and the pooled cohort study published by Marsh et al. (Marsh et al. 2017b) were taken as key studies for the evaluation of human carcinogenicity data for the Co OEL.

Experimental data

Several animal studies investigated the induction of tumours following chronic exposure to cobalt compounds. Out of them, we agree with the EPA that only the two 2-year inhalation carcinogenicity studies with cobalt sulfate hexahydrate and with Co metal powder in rats and mice by Bucher et al. (NTP) are considered adequate to assess the carcinogenic potential of cobalt ions (Behl et al. 2014; Bucher et al. 1998). Chronic inhalation exposure of rats and mice to aerosols of cobalt sulphate hexahydrate resulted in increased incidences of alveolar/bronchiolar neoplasms in both sexes of both species at concentrations ≥ 300 µg/m^3 cobalt sulphate hexahydrate (equivalent to ≥ 67 µg Co/m^3). Significantly increased rates of lung tumours (alveolar/bronchiolar carcinoma) were seen in all rats (males and females) exposed to ≥ 1,250 µg Co/m^3 as compared to controls. In mice, significantly increased incidences of alveolar/bronchiolar carcinoma were found as well in all exposed male and female mice as compared to controls (Co metal powder study).

There is “clear evidence” (NTP terminology) for the carcinogenicity of Co sulphate (hydrated form) in female rats and male and female mice, with “some evidence” in male rats. There is “clear evidence” from the NTP inhalation studies that cobalt metal is carcinogenic in both sexes of the two species, rat and mouse.

A NOAEC for formation of lung tumours was not reached in these studies, however, the data are suitable for the postulation and modelling of a dose response, in which the Co sulphate data are closer to the LOAEL/modelled NOAEL section of the dose response curve.

Cancer dose-response

For the endpoint cancer, no dose response assessment in humans (using the epidemiology data) can be conducted due to the lack of an observed effect (i.e. no increase of cancer incidence) at the exposure experienced in the cohorts. Due to the significant size of the cohort, which is approximate to the number of workers employed in the primary cobalt industry in the EU, this paper considers these data relevant to the low end of the dose response curve and will include knowledge from these cohorts as a qualitative consideration in the dose response assessment. Regarding experimental animal studies, for the endpoint lung cancer, no NOAEC has been determined (chronic inhalation exposure to cobalt substances). However, the effect level forms a dose response that can be used to model dose responses also at lower risk/exposure which is relevant for humans. The human data should be used as a “plausibility

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
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check" to compare the NOAEC as modelled from the animal data with the NOAEC observed in the human cohorts. The two data sets (human and rodent) are plotted together to provide a qualitative picture of exposures and presence of a threshold for cancer (see Figure 4).

Figure 4 can be reconciled with the presence of a threshold for cancer at exposure concentrations in the region between 20 and 80 µg Co/m^3 (time-weighted average; TWA), and a flat trend of the dose-response relationship from a position within that window. In the absence of an empirical no-observed adverse effect concentration (NOAEC) for cancer, the "breakpoint" in the dose response curve can be interpreted as the threshold of response (cancer), below which no increased risk occurs. This interpretation is based on the lack of observed in response in the large cohorts exposed to Co, and with no demonstratable elevated probability of response (cancer). As a dose response is only present in the rodent studies, the "breakpoint" must be calculated from rodent data.

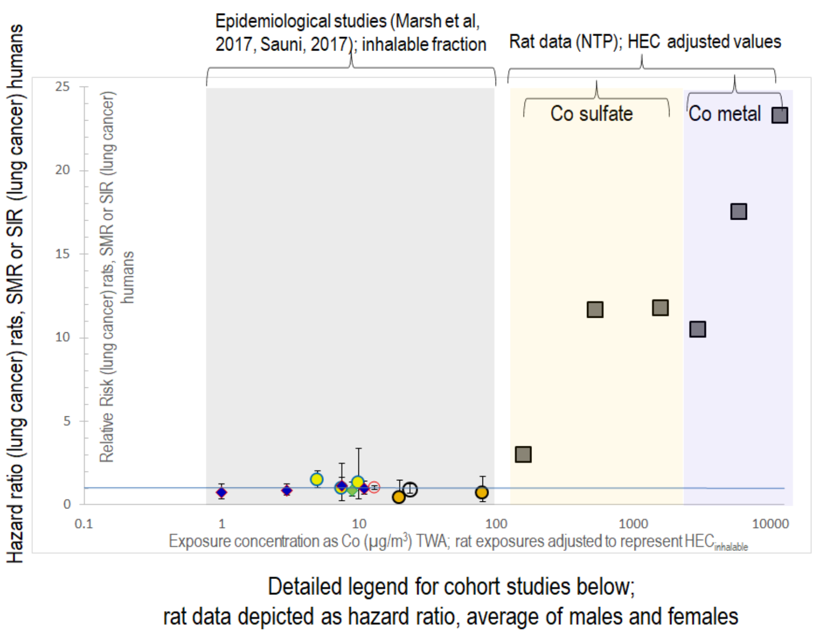

Figure 4. Cancer dose-response across species (adjusted to represent human equivalent concentration).

Legend: Human data points: different country cohorts, long-term exposures (> 5 years) only. Average exposures (time weighted averages) as reported by Marsh et al, 2017 and Sauni et al, 2017 are shown. Y-axis represents the SMR (Standardised Mortality Ratio) for the epidemiological data, and a "relative risk" calculated as "cancer incidence exposed" / "cancer incidence unexposed" in male and female rats combined. Rodent exposure concentrations are adjusted to reflect the Human Equivalent Concentration, (i.e NTP rodent inhalation studies exposure concentrations multiplied by HEC). Human exposure concentrations are shown as TWA.

Detailed legend for epidemiology studies (human data points) below:

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Mean exposure as TWA (µg Co/m^3)	Symbol, SMR	CI	
Swedish cohort, 1year and longer workers			From table 8 of Swedish cohort mortality study (Westberg et al, 2017), cumulative exposure divided by 40 years. Co exp classes (total), of the brackets the midpoints were chosen for plotting; e.g. for the bracket 0,21 - 0,4 mg/m3, the midpoint was chosen as 0,305 and divided by 40.
< 5	1,48	1.05-2.02	
7,5	0,96	0.26-2.46	
10	1,33	0.36-3.4	
US complete cohort, short term and long term workers			From table 10 of US cohort (Marsh et al, 2017), AIE (Average Intensity exposure, as these are CUM exposures divided by actual years worked). For each exposure bracket, the midpoint was chosen for plotting, e.g. bracket 0,005-0,010 mg/m3 is represented by 7.5 µg Co/m3.
< 1	0,73	0.38-1.28	
3	0,87	0.59-1.24	
7,5	1,16	0.78-1.65	
11	0,99	0.65-1.44	
German cohort, complete cohort			Took mean of low backward extrapolation based on JCN-JEM and SEG-JEM cum.exposure Co Mean value (Table 3). 160 + 117 ug Co/m3/year = average 139 ug Co/m3. Divided by actual duration of work (5.9 years).
23,5	0,93	0.68-1.25	
Austrian cohort, complete cohort			Exposure data from text in Wallner et al, 2017 page e285, left column. CUM exposure mean of 0,52 was divided by 40 (years of exposure). Relative Risk as Hazard Ratio HR and CI from table 2. Risk by CUM exposure.
13	1,03	0.94-1.13	
UK cohort			Median exposure table 1 of McElvelnny et al, 2017, "all data". Data are given as mg/m3, no CUM exposures available. SMR taken from table 4, comparison with England, Wales and West Midlands is the same with 0,85.
9	0,85	0,54-1,34	
Finnish cohort			SIR for risk of lung cancer is given. Sauni et al 2017, table 4 SIRs for low and high exposure groups. Exposure TWAs corresponding to "high" and "low" exposure groups are taken from graph Fig. 2
20	0,41	0.05-1.47	
80	0,67	0.18-1.72	

2

The approach to estimate the probability of response from a comprehensive database, including studies from different species and even on different adverse endpoints, is described in detail by Chambers et al. (2010). The severity scoring applied by Chambers et al. in their categorical regression study is comparable to the assignment of “relative risk” of lung cancer in the approach described below. In contrast to the work by Chambers et al. (2010), the data below are used for visualization purposes only, and are not used to model a dose response across different species.

In particular, the figure is presented for the visualisation of several points:

- The shape of the exposure-response curve is flat at low exposures and shows an increasing trend at the exposure concentrations applied in the rodent studies.
- It appears that humans are <u>not</u> more sensitive to cobalt than rats.
- It appears that the threshold for the carcinogenic response is likely to be in the narrow window between highest cohort exposure and lowest rodent exposure.
- Due to uncertainties in the combination of inhalation data from different species, and due to lack of a dose response within the human data alone, the calculations will be carried out with the rodent data (rats and mice) only.
- A non-linear assumption should be applied to model the dose response of the rodent data (calculation of a benchmark dose).

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Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
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- The breakpoint in the curve may, however, be set on the highest human exposure experienced in the human cohorts. This could be the reported highest time weighted average, or the highest reported cumulative exposure, adjusted for work lifetime (40 years).

Alternatively, the breakpoint in the curve may be set on the benchmark dose (BMD), modelled lower confidence limit of the BMD10 (i.e. BMDL10) calculated from the rodent data.

The human equivalent concentration (HEC) was used solely to adjust the respirable rodent data from the NTP carcinogenicity studies into inhalable values for a direct comparison to the epidemiological data (inhalable fraction). For the unadjusted figure, please see below.

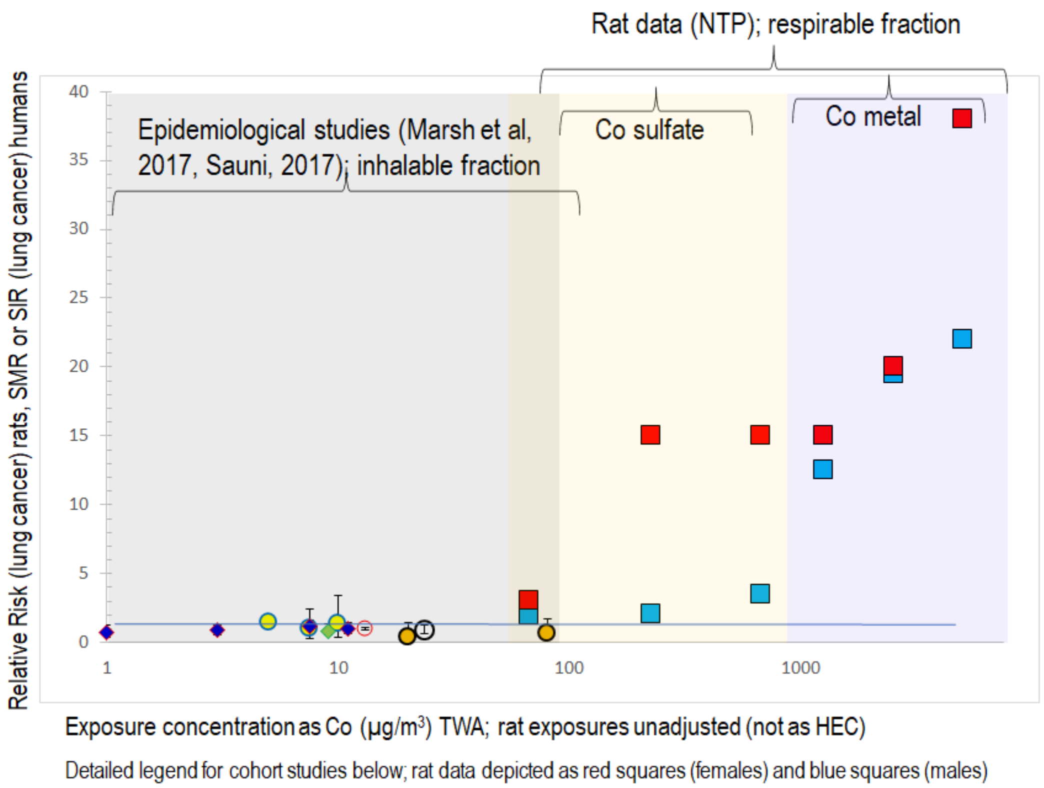

Figure 5. Cancer dose-response across species (unadjusted)

4. Independence of tumors in Co NTP studies

Summary

- Several aspects cast doubt on the interpretation that the individual systemic tumors are independent and directly related to cobalt.
- The predominant systemic finding (adrenal pheochromocytoma) is a well-known response to respiratory distress and hypoxia.
- For the remaining systemic tumors:
  - There is a lack of an exposure-response relationship
  - They occurred only in one sex (either males or females) of the rats
  - There is a complete lack of a historical control database for this rat colony (F344/NTac), making it impossible to conclude whether the systemic tumors are biologically relevant or statistically significant
  - This rat colony is uniquely sensitive and had developed a number of spontaneous diseases that immediately (after one inhalation study) led to the discontinuation of the use of this colony at NTP

There were exposure-concentration dependent increases in the incidences of benign and malignant pheochromocytoma (combined) in all substance-exposed male and female rats. This effect was not observed in mice. These tumors are well-established responses that are secondary to hypoxia and respiratory distress in rats (Ozaki et al. 2002).

In a statistical re-evaluation of nine 2-year NTP inhalation studies, a range of lung effects chronic active inflammation, interstitial fibrosis, alveolar epithelial hyperplasia, squamous metaplasia, proteinosis, and histiocytosis) and their association with pheochromocytoma was investigated. It was concluded that there is an overall association between lung impairment *by any cause* and an elevated incidence of adrenal pheochromocytoma in NTP inhalation studies. The elevated incidences of pheochromocytoma in rats after inhalation exposure to Co metal are considered to be rat specific responses to respiratory distress, with no causal relationship to Co. Also, there is no indication for an involvement of genotoxic mechanisms in the induction of pheochromocytoma by chemicals in animals (Ozaki et al., 2002; Greim et al., 2009).

Therefore, these tumors should not be assumed to be occurring independently, as this is not supported by the MOA leading to pheochromocytoma in inhalation studies, and may lead to a severe overestimation of the potency of Co ion related carcinogenicity.

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: ci@cobaltinstitute.org
www.cobaltinstitute.org

The assumption of independence of the tumors warrants a closer look at all tumorigenic findings in the NTP inhalation studies with Co sulfate and Co metal powder.

Rare systemic tumors in the context of historical control data

Historical control data are needed to decide whether a tumor is “rare” (background rate of < 1%) or “common” (background rate > 1%) and are needed to interpret the significance especially of rare tumors and of marginally increased tumor incidences. In the NTP Co metal inhalation study, the tumors in kidney and pancreas can probably be considered “rare”, however, in this context, it needs to be outlined that there are <u>no historical control data for the F344/NTac strain</u> (the F344/N colony at Taconic laboratories) and inhalation exposure route (in that strain) at NTP. In total, only two carcinogenicity studies were carried out at NTP with the F344/NTac rats, one by inhalation (the Co metal study) and one by p.o. route of exposure (TR 583, Bromodichloroacetic Acid, drinking water study). The “historical control” used by the NTP in the Co metal report consisted of only 100 animals, which actually *includes the concurrent control* (50 animals), with the addition of another 50 animals of study TR 583, exposed by a different route of exposure. This is not what would constitute a “historical control”. For comparison, a typical historical control database would consist of around 50 studies by the same route of exposure, and several thousand animals (Deschl et al., 2002).

Lack of historical control data for the Fisher F344/NTac rat strain

Only one inhalation carcinogenicity study was ever conducted at the NTP with the F344/NTac rat – with cobalt metal powder. It is important to realize that the F344/NTac rats had developed a number of problems specific to this colony, including “declining fertility, sporadic seizure activity, and chylothorax” (King-Herbert and Thayer, 2006).

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

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

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
www.cobaltinstitute.org

Common systemic tumors: Mononuclear cell leukemia (MNCL)

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

MNCL occurs with a high spontaneous background rate, and occurred at 42% and 36% in the controls, males and females, respectively. The incidence of MNCL is high across all exposure groups in the male rats, including controls (42%, 50%, 44%, 44% in control, 1.25, 2.5 and 5 mg Co/m^3 exposure groups, respectively); it is also high in all female rats with 36%, 62%, 61%, 59% in control, 1.25, 2.5 and 5 mg Co/m^3 exposure groups, respectively. The female control animals display an in fact somewhat low incidence of MNCL. These data reflect the general observation that MNCL is a common tumor type, and that Fisher rats are generally prone to developing MNCL as they age (Suckow, Weisbroth and Franklin, 2006). Extremely elevated incidences of MNCL have been previously observed in a number of chronic bioassays and 2-year carcinogenicity studies in F344 rats (Haseman et al., 1998; Caldwell, 1999). The analysis of the spontaneous neoplasm incidences in F344 rats from chamber controls of 18 two-year inhalation studies carried out by the NTP revealed a frequent occurrence of MNCL in males (57.5%, range 34-70%) and in females (37.3%, range 24-54%) [9]. The data show that MNCL occurs in untreated aged rats at extremely high and variable rates. The conclusion that MNCL is a Co related tumor based on the data in *female* rats cannot be substantiated when taking into account the data from both sexes, and when taking into account the high and variable occurrence of this common tumor.

MNCL is uncommon in most other rat strains, and its background incidence in the Fisher rat has increased significantly over time. MNCL has not been found in other mammalian species and no histologically comparable tumor is found in humans (Caldwell 1999). In the light of the well-known occurrence of MNCL in the Fisher rat, this result does not suggest that this is an independently occurring tumor directly related to Co exposure.

Kidney, adenoma/carcinoma combined

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

The neoplasms in the kidney were slightly above the concurrent control data, but not statistically significant and no overall positive trend was established. In the light of these

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
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arguments, these findings do not appear to warrant an assumption that these tumors are independently occurring and related to Co exposure.

Pancreatic islets

There was a small increase in islet-cell tumors in the mid- and high-dose male rats but not in female rats (a small but not statistically non-significant increase was seen in the highest dose group). Mice did not display this effect. These tumors are rare and they were seen for the first time in an NTP study. Also, the F344 NTac rat was used for the first, and only, time in an NTP inhalation study. It is impossible to interpret these findings, and the statement in the NTP report that there was “equivocal evidence of carcinogenic activity” is considered justified. This level of evidence should not be taken as a basis for a conclusion that these are independently occurring tumors caused by exposure to Co.

Apart from the pheochromocytoma occurring in the Co sulfate study, systemic tumors were observed exclusively in the inhalation study with Co metal powder. This may be related to the very high exposure concentrations (adjusted for Co equivalent, the lowest dose in the Co powder study was higher than the highest dose in the Co sulfate study), or it may reflect the health issues that have led to the immediate discontinuation of the use of the F344/NTac colony in NTP cancer bioassays.

18 Jeffries Passage • Guildford • GU1 4AP • UK
Tel: +44 1483 578877 • Fax: +44 1483 567042 • Email: vviegas@cobaltinstitute.org
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5. Stantec ChemRisk Attachment

We have provided the introduction/summary for Attachment 1 below:

In the IRIS Assessment Plan and Protocol for Cobalt and Cobalt Compounds, the EPA identified several key scientific issues for consideration when assessing hazard and dose response for cobalt and cobalt compounds. Such issues include: Tumor dependencies; cellular uptake and tissue disposition; cobalt particle toxicity; proposed mode of action (MOA) of cobalt carcinogenicity; and the diversity of substances included in the group. Cobalt has been evaluated by several other agencies, particularly with respect to carcinogenicity and dose response, including California's Office of Environmental Health Hazard Assessment (OEHHA), the Texas Commission for Environmental Quality (TCEQ) and the European Chemicals Agency (ECHA). EPA has indicated that "these cobalt-related science issues will be informed by conclusions from prior assessments" and thus we believe it is essential that EPA appreciate the strengths and weaknesses of these existing assessments in evaluating cobalt and cobalt compounds under IRIS. This report provides a brief review of the approaches relied upon in these assessments focused on carcinogenicity and associated dose response, with some evaluation for EPA's consideration in their approach to evaluating cobalt and cobalt compounds.

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Annex 1. Properties of 12 cobalt substances

Table 1. Characteristics of 12 cobalt substances (as taken from Verougstraete et al. 2022)

Table 1
Characteristics of the twelve cobalt substances.

Name	Formula	CAS	%Co	Surface area (SA) (m^2/g)	D50 (μm)	Water solubility at 20 °C (mg Co/L)	Bioaccessibility study
Cobalt dichloride	$CoCl_2$	7646-79-9	25.0	0.78	–	145,900	#1
Cobalt dinitrate	$Co(NO_3)_2$	10141-05-6	20.3	0.32	–	135,600	#2
Cobalt sulphate	$CoSO_4$	10124-43-3	21.0	3.49	–	124,700	#1
Cobalt di(acetate)	$Co(CH_3COO)_2$	71-48-7	23.5	3.28	–	82,350	#2
Cobalt sulphide	CoS	1317-42-6	63.0	218.19	26.24	9.7	#3
					26.24		#4
Cobalt carbonate	$CoCO_3$ (#1)	513-79-1	45.0	103.05	–	5.92	#1
	$CoCO_3$ (#3)			19.93	10.25		#3
Cobalt oxide	CoO (#1)	1307-96-6	78.5	4.79	–	4.12	#1
	CoO (#3)			15.56	5.24		#3
Cobalt	Co (#1)	7440-48-4	100.0	1.20	–	2.94[a]	#1
	Co (#3)			3.75	4.58		#3
Cobalt dihydroxide	$Co(OH)_2$	21041-93-0	61.9	12.95	19.18	1.42	#2
Tricobalt tetraoxide	Co_3O_4 (#2)	1308-06-1	72.4	4.70	–	1.17	#2
	Co_3O_4 (#3)			3.16	6.65		#3
Cobalt hydroxide oxide	CoOOH	12016-80-7	67.2	76.72	12.63	0.45	#2
Lithium cobalt dioxide	$CoLiO_2$	12190-79-3	60.2	0.20	12.16	0.18	#2

#1: results are reported in Turner (2002) and Stopford et al. (2003)..
#2: results from Kirby reports 2008.
#3: results from Kirby reports 2013.
#4: result from ECTX report 2021.
[a] Is a measure of corrosion rather than water solubility.

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Table 2. Bioaccessibility data from cobalt substances in three lung fluids ranked by water solubility high to low (as taken from Verougstraete et al. 2022).

Table 3A

Bioaccessibility data from cobalt substances in three lung fluids ranked by water solubility high to low; all data correspond to a loading of 2 g/L. Cobalt releases are expressed as mg Co/g sample.

Rank	Substance (ranked by water solubility)	"short extraction time" 5 (or *2*)* hours						24 h						72 h					
		AIF mg Co/g sample		AAF mg Co/g sample		ALF mg Co/g sample		AIF mg Co/g sample		AAF mg Co/g sample		ALF mg Co/g sample		AIF mg Co/g sample		AAF mg Co/g sample		ALF mg Co/g sample	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
1.	CoCl2	*120.05*	*21.57*	*128.35*	*22.98*	*223.00 n.a.*	*1.84*	171.70	2.40	*n.a.*	-	*n.a.*	–	195.50	7.07	*n.a.*	–	*n.a.*	–
		114.20	5.66	169.98	13.60		-												
2.	Co(NO3)2	42.20	3.96	32.19	2.21	204.0	0.68	48.83	1.59	45.38	1.33	*n.a.*	–	131.45	6.13	57.20	1.40	*n.a.*	-
3.	CoSO4	*146.25*	*1.48*	*105.50*	*1.27*	*165.40 n.a.*	13.86	142.90	11.03	*n.a.*	–	*n.a.*	–	173.80	0.14	*n.a.*	–	*n.a.*	–
		139.00	5.52	108.07	18.57														
4.	Co(CH3COO)2	22.94	0.98	24.51	0.06	181.47	14.81	25.87	0.84	28.31	3.17	184.50	4.24	39.36	1.70	27.12	2.43	198.10	9.47
5.	CoS	0.61	0.01	5.62	0.21	3.68	0.09	1.02	0.01	7.93	0.80	6.64	0.07	1.97	0.31	8.79	0.94	14.72	0.27
6.	CoCO3 (#1)	*7.98*	*0.12*	*6.23*	*1.25*	*431.75 n.a.*	*0.35*	5.44	0.75	6.84	0.85	*n.a.*	–	7.11	0.27	13.07	0.84	*n.a.*	-
		9.68	2.51	5.63	0.87		-												
	CoCO3 (#3)	*n.a.*	–	1.11	0.08	539.05	6.02	*n.a.*	–	3.72	0.04	544.35	4.76	*n.a*	–	5.12	0.08	525.27	15.03
7.	CoO (#1)	*4.70*	*0.08*	*6.28*	*0.78*	*720.45*	*5.44*	14.63	9.57	7.74	0.52	702.20	9.62	77.30	20.79	19.71	6.53	*n.a*	–
		12.30	1.98	7.04	0.06	653.50	0.71												
	CoO (#3)	*n.a.*	–	2.08	0.01	689.83	2.15	*n.a.*	–	12.84	0.21	781.73	7.80	*n.a.*	-	19.45	0.84	813.48	10.19
8.	Co (#1)	*33.36*	*0.23*	*9.58*	*1.98*	*910.25 n.a*	11.67	35.04	4.09	17.57	1.03	*n.a*	–	40.14	8.44	47.71	9.12	*n.a*	–
		37.46	1.71	12.37	0.12		–												
	Co (#3)	*n.a*	–	28.10	0.38	953.25	21.05	*n.a*	–	29.62	1.93	966.06	19.44	*n.a*	-	39.41	0.20	984.41	27.58
9.	Co(OH2)	1.79	0.12	1.43	0.02	607.02	19.60	3.39	0.14	3.55	0.30	*n.a*	–	10.15	0.33	22.43	0.74	*n.a*	-
10.	Co3O4 (#2)	0.03	0.00	0.03	0.00	20.36	0.27	0.03	0.00	0.03	0.00	23.36	0.17	0.06	0.00	0.03	0.00	30.68	0.65
	Co3O4 (#3)	*n.a.*	–	0.05	0.00	1.38	0.04	*n.a*	-	0.05	0.00	2.73	0.03	*n.a*	-	0.26	0.01	5.30	0.33
11.	CoOOH	0.10	0.02	0.05	0.00	33.92	1.02	0.10	0.01	0.19	0.00	327.39	3.09	0.10	0.00	0.32	0.01	495.04	10.55
12.	CoLiO2	0.03	0.00	0.03	0.00	7.68	0.14	0.03	0.00	0.03	0.00	20.22	0.35	0.05	0.01	0.05	0.01	45.03	1.04

n.a.: data not available.

SDs are calculated on the mean of the duplicates (studies #1, #2, 3) or triplicates (study#4). SDs exceeding 20% are indicated in bold.

* Values given are for 5 h and when 2h values are also available they are listed in italics above the 5h values.

18 Jeffries Passage • Guildford • GU1 4AP • UK
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