IRIS PUBLIC SCIENCE MEETING

January 11, 2023

Welcome and Logistics

- Keep your phone **muted** throughout the Zoom Meeting.
- **To ask a question or provide a comment**, use the "Chat" pod of Zoom Meeting to inform the meeting host of your question. Questions and comments (webinar) will be posed at the end of each issue discussion.
- **To report technical difficulties or webinar issues to the meeting host,** use the "Chat" pod of the Zoom Meeting.

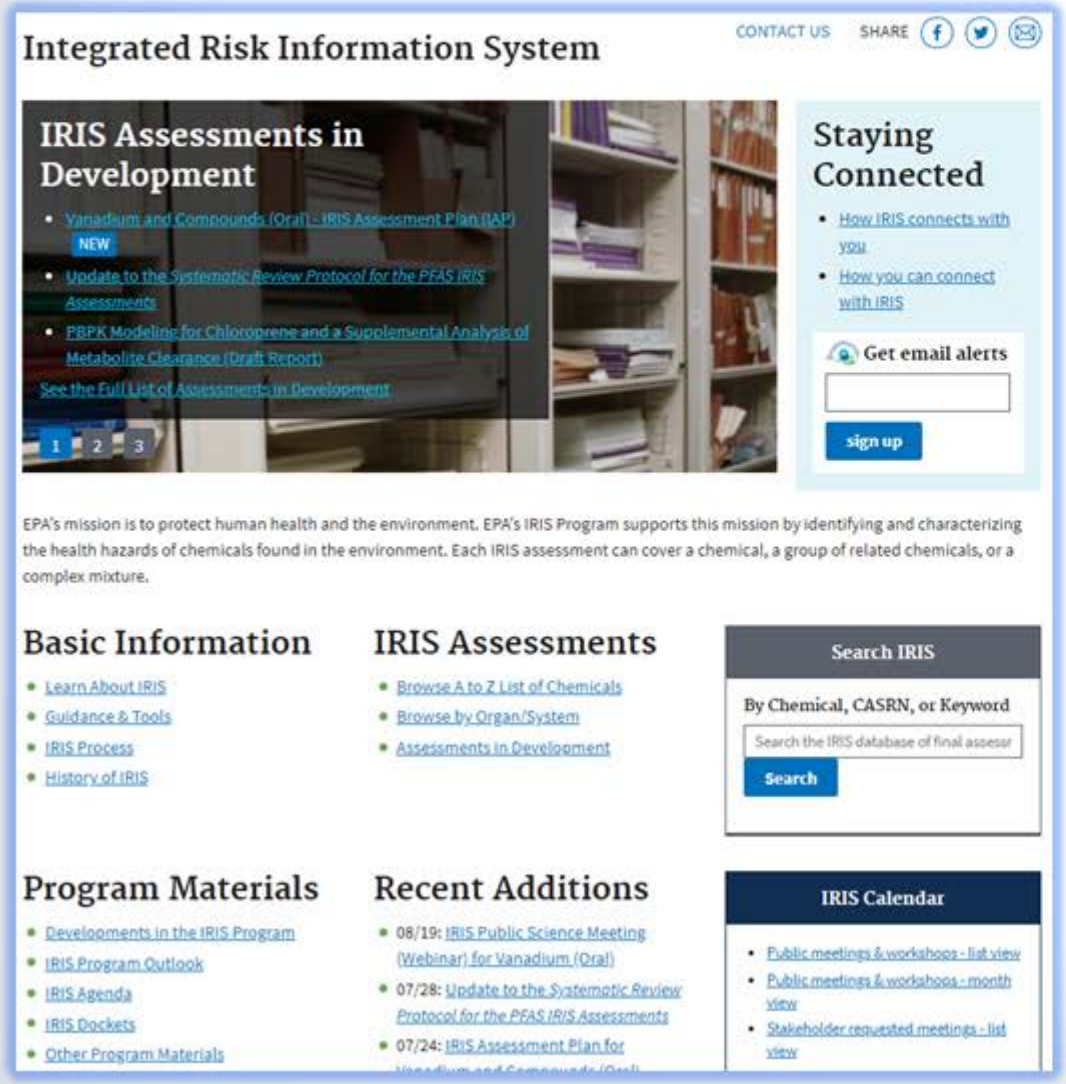
INTRODUCTION AND ROLE OF ASSESSMENT PLANS IN THE IRIS PROCESS

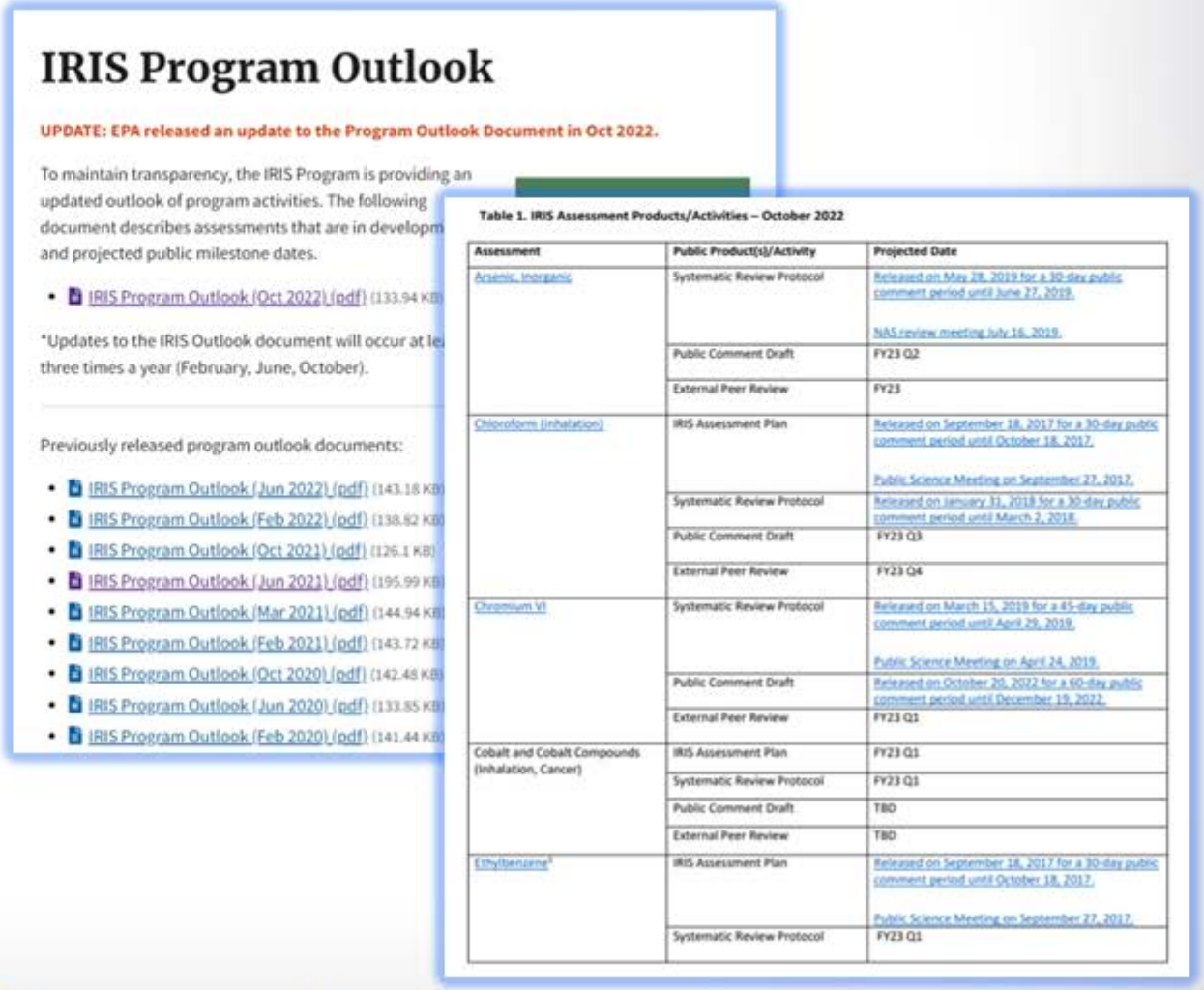
Kris Thayer
Director, Chemical & Pollutant Assessment Division (CPAD)
Center for Public Health and Environmental Assessment (CPHEA)
Office of Research and Development
U.S. Environmental Protection Agency

- **IRIS assessments contribute to decisions across EPA and other health agencies.**

- **Toxicity values**
  - Noncancer: Reference Doses (RfDs) and Reference Concentrations (RfCs).
  - Cancer: Oral Slope Factors (OSFs) and Inhalation Unit Risks (IURs).

- **IRIS assessments have no direct regulatory impact until they are combined with**
  - Extent of exposure to people to determine risk
  - Regulatory options given applicable statutes, cost of cleanup, available technology, etc.
  - These are the purview of EPA's program offices





https://www.epa.gov/iris

IRIS Program Outlook

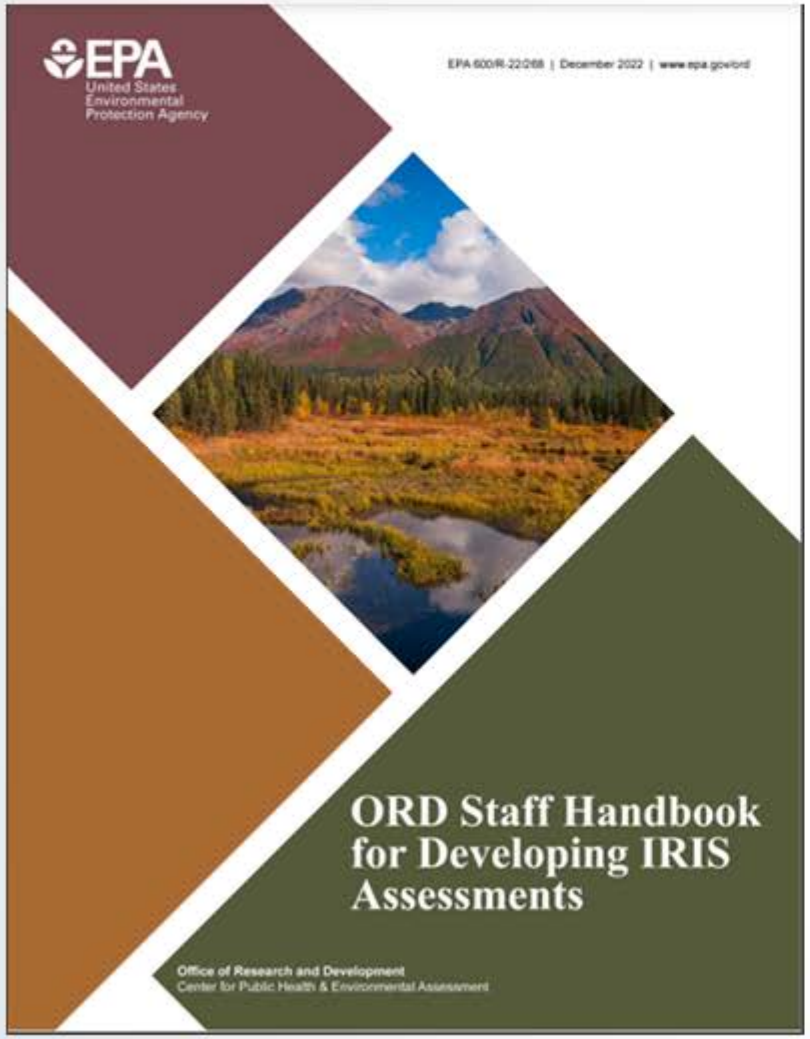
- To maintain transparency, ORD has developed a public IRIS Program Outlook.
- Describes assessments that are in development and projected public milestone dates.
- Updates to the IRIS Outlook document occurs at least three times a year (February, June, October).
- **Cobalt added to the IRIS Program Outlook in June 2022.**
- IAP and protocol released Nov 2022

Table 1. IRIS Assessment Products/Activities – June 2022

Assessment	Public Product(s)/Activity	Projected Date
Arsenic, Inorganic	Systematic Review Protocol	Released on May 28, 2019 for a 30-day public comment period until June 27, 2019. NAS review meeting July 16, 2019.
	Public Comment Draft	FY23 Q2
	External Peer Review	FY23
Chloroform (Inhalation)[1]	IRIS Assessment Plan	Released on September 18, 2017 for a 30-day public comment period until October 18, 2017. Public Science Meeting on September 27, 2017.
	Systematic Review Protocol	Released on January 31, 2018 for a 30-day public comment period until March 2, 2018.
	Public Comment Draft	FY23 Q1
	External Peer Review	FY23 Q2
Chromium VI	Systematic Review Protocol	Released on March 15, 2019 for a 45-day public comment period until April 29, 2019. Public Science Meeting on April 24, 2019.
	Public Comment Draft	FY23 Q1
	External Peer Review	FY23 Q1
Cobalt and Cobalt Compounds (Inhalation, Cancer)[2]	IRIS Assessment Plan	FY23 Q1
	Systematic Review Protocol	FY23 Q1
	Public Comment Draft	TBD
	External Peer Review	TBD
Ethylbenzene[2,3]	IRIS Assessment Plan	Released on September 18, 2017 for a 30-day public comment period until October 18, 2017. Public Science Meeting on September 27, 2017.
	Systematic Review Protocol	FY23 Q1

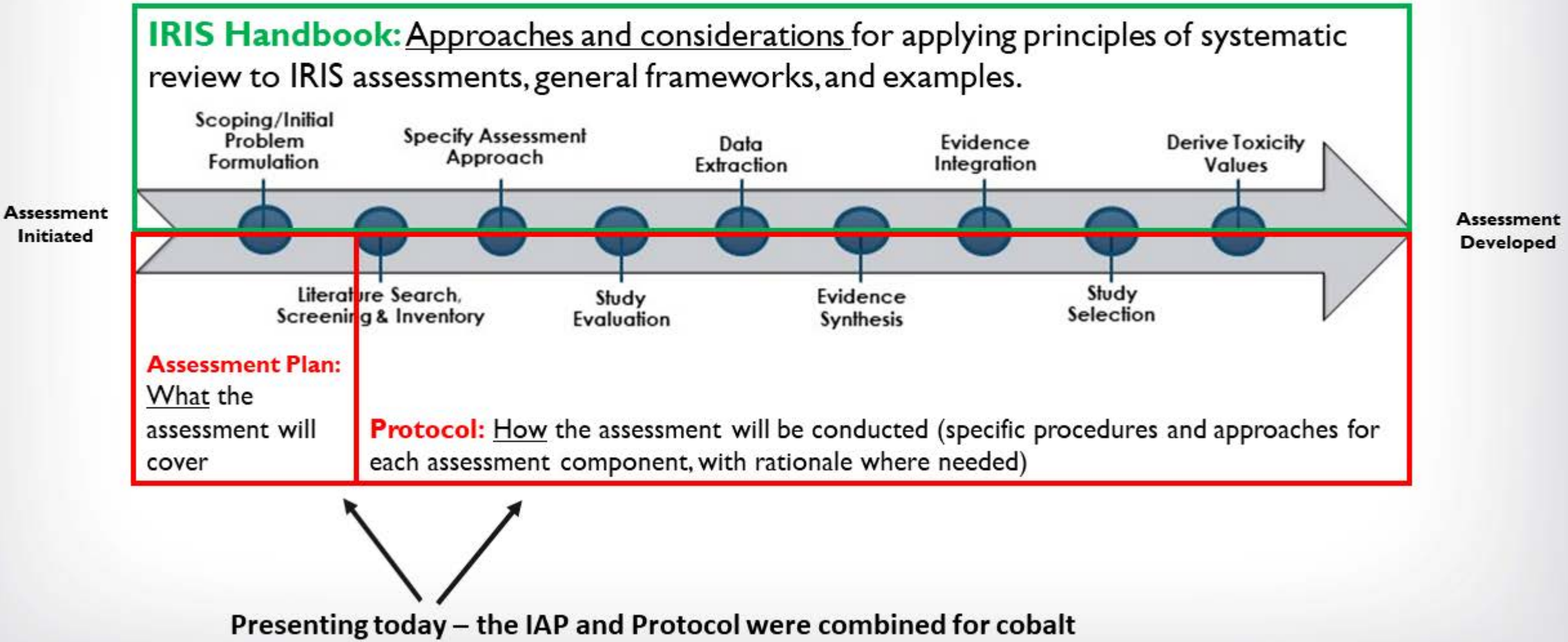
https://www.epa.gov/iris/iris-program-outlook

IRIS Handbook



- Released December 22, 2022
- Handbook covers systematic review and dose-response methods
- Reviewed by National Academy of Science Engineering and Medicine (NASEM November 2021)
- Primary intents are to:
  - Increase transparency
  - Foster consistency in assessments developed by the IRIS Program

IRIS Systematic Review Documents



Presenting today – the IAP and Protocol were combined for cobalt

IRIS Assessment Plan and Protocol for Assessing Cancer Risk from Inhalation Exposure to for Cobalt and Cobalt Compounds

January 11, 2023

Alan Sasso, PhD
Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency

The purpose of this IRIS Public Science Meeting is to discuss the science that informs the Public Comment Draft IRIS Assessment Plan and Protocol for Assessing Cancer Risk from Inhalation Exposure to Cobalt and Cobalt Compounds. The draft plan and this presentation do not represent and should not be construed to represent any Agency determination or policy.

Background

- Naturally occuring element
  - Largest deposits are in Alaska, California, Idaho, Maine, Michigan, Minnesota, Missouri, Montana, North Carolina, New Mexico, Oregon, Pennsylvania, Puerto Rico and Tennessee (source: USGS)
- Used in a variety of industrial applications
  - Colorant for glass, ceramic, and paint
  - Catalysts, batteries
  - Production of hard-metals, metal alloys
  - Internal metal prosthetics
- Present at 351 active Superfund sites
  - Landfills, mines, metal plating facilities, military bases and shipyards

Program Office Interest

- "Cobalt compounds" are listed as a hazardous air pollutant
- November 2021, EPA's Office of Air and Radiation (OAR) nominated water-soluble and water-insoluble cobalt compounds for an inhalation cancer assessment
- OAR's priority need is to inform risk determinations, for regulation under the Clean Air Act (CAA), impacted by potential carcinogenicity from air emissions of cobalt compounds. These arise from industrial processes and cobalt compounds have been identified from current emission data.
- The IRIS database currently does not contain a cancer classification or unit risk for cobalt.

Previous Assessments

- EPA 2008 Provisional Peer Reviewed Toxicity (PPRTV) assessment: water-soluble cobalt compounds are "likely to be carcinogenic to humans by the inhalation route"
- NTP, IARC, California EPA, and Texas CEQ have also concluded that cobalt and certain cobalt compounds are likely to cause cancer.
  - CalEPA, TCEQ, and EPA PPRTV derived unit risks for cobalt

Scope of Assessment

- Focus will be inhalation exposure
- Focus on cobalt forms most pertinent to implementing the CAA
- Out of scope:
  - Non-bioavailable forms (i.e., Vitamin B12)
  - Radioactive forms (i.e., ^{60}Co)

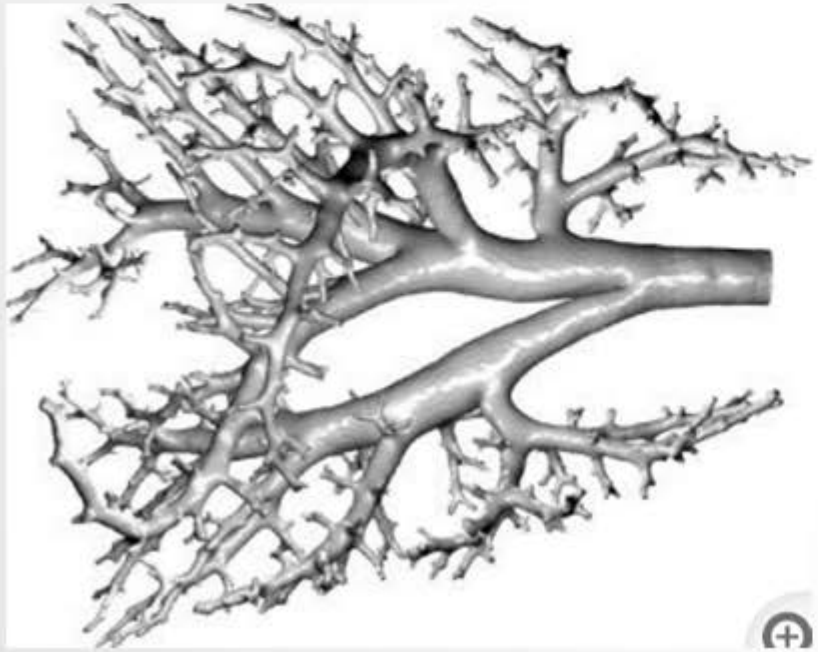
Specific Aims

- **Objective** – develop cancer inhalation unit risks (IURs) for water-soluble and water-insoluble cobalt compounds
  - Adopt EPA's PPRTV cancer hazard conclusion that under *EPA's Guidelines for Carcinogen Risk Assessment*, cobalt is "likely to be carcinogenic to humans by the inhalation route"
  - Evaluate mechanistic and ADME information to inform dose-response
  - Conduct inhalation dosimetry and dose-response modeling
- Utilize a systematic evidence map (SEM) to identify studies most suitable for deriving IUR(s)
  - SEM built of other assessments (RoC, CalEPA, ATSDR, TCEQ, PPRTV).

Specific Aims

EPA's Multiple-Pathway Particle Dosimetry (MPPD) model, in conjunction with other approaches, will integrate physicochemical properties (size, distribution, density) with physiology to account for interspecies differences

Rat



Human

PECO: Populations, Exposures, Comparators, and Outcomes

Populations	**Human:** Any population and lifestage (occupational or general population, including in pregnant women, infants, children, adolescents and adults). **Animal:** Nonhuman mammalian animal species (whole organism) of any lifestage (including fetal, early postnatal, adolescents and adults). Studies of transgenic animals are tracked as mechanistic studies under "potentially relevant supplemental material". *Note: Studies meeting PECO criteria may also contain information on susceptible populations. When this occurs, these studies are also tagged as having information pertinent to susceptible populations. This typically happens during preparation of the literature inventory or full text extraction.*
Exposures	**Relevant forms for Clean Air Act:** cobalt aluminate (1345-16-0), cobalt bromide (7789-43-7), cobalt carbonate (513-79-1), cobalt carbonyl (10210-68-1), cobalt chloride (7646-79-9), cobalt (7440-48-4), cobalt hydrocarbonyl (16842-03-8), cobalt naphtha (61789-51-3), cobalt nitrate (10141-05-6), cobalt oxide (1307-96-6), cobalt oxide (II, III) (1308-06-1), and hexanoic acid, 2-ethyl-, cobalt(2+) salt (136-52-7). Many of these compounds do not have cancer toxicity information, thus other water-soluble and water-insoluble cobalt compounds that do have inhalation cancer evidence are included within the scope of this review, e.g., cobalt sulfate, cobalt hydroxide, and cobalt sulfide. *Radioactive isotopes (i.e., ^{60}Co) and vitamin B12 are considered out of scope.* **Human:** Any quantitative exposure to cobalt via the inhalation route, aside from acute or very short (days) duration. Studies of developmental exposure are also included. Studies will also be included if biomarkers of exposure are evaluated (e.g., measured compound or metabolite levels in tissues or bodily fluids) and the exposure route can be inferred as primarily inhalation. **Animal:** Any quantitative exposure to cobalt via the inhalation route for any subchronic and chronic exposure duration. Studies of developmental exposure are also included. Studies involving exposures to mixtures will be included only if they include exposure to a relevant form of cobalt alone. Non-inhalation routes, including oral, dermal or intravenous, are tracked as "potentially relevant supplemental information."
Comparators	**Human:** Referent populations exposed to lower (within the study) levels of cobalt. The results of the comparisons must be presented with sufficient detail of quantitative modeling (e.g., regression coefficients presented with statistical measure of variation). *Case reports describing findings in 1-3 people are tagged as "potentially relevant supplemental information."* **Animal:** A concurrent control group exposed to vehicle-only treatment and/or untreated control.
Outcomes	Any **cancer-related** effect on any system.

Focusing the Analysis

- Epidemiological and animal toxicology studies included after full-text review for meeting PECO criteria were evaluated for suitability for dose-response (SEM Appendix, Section 8.7)
  - For animal studies, the analysis focused primarily on study design features
  - For epidemiological studies, observations on study limitations (risk of bias/sensitivity) from the RoC monograph were cited
  - Some studies outside of our search criteria (identified in public comments) are being screened according to PECO
- NTP inhalation cancer bioassay studies for cobalt sulfate (NTP, 1998) and cobalt metal (NTP, 2014) were considered the best suited for dose-response analysis

Key Science Issues

Science Topic 1: Association between lung and adrenal tumor formation

- An analysis of the results of NTP* inhalation exposure studies in rats found an apparent association between the occurrence of pulmonary non-neoplastic lesions and the development of pheochromocytomas. This plausible association has been attributed to the adrenal response arising from systemic hypoxemia due to the reduced gas exchange induced by the lung lesions and the accompanying fibrosis and chronic inflammation.
- Assessment of the dependence of the tumor types impacts the method used to estimate composite cancer risk. A combined tumor analysis may not be appropriate if tumors do not form independently.

*NTP (National Toxicology Program). (1991). Toxicity studies of cobalt sulfate heptahydrate 41 (CAS no 10026-24-1) in F344/N rats and B6C3F1 mice (inhalation studies). (NIH 42 Publication No. 91-3124). Research Triangle Park, NC.

*NTP (National Toxicology Program). (1998). Toxicology and carcinogenesis studies of 44 cobalt sulfate heptahydrate (CAS No. 10026-24-1) in F344/N rats and B6C3F1 mice 45 (inhalation studies). (NTPTR471). Research Triangle Park, NC.

*NTP (National Toxicology Program). (2014). NTP Technical Report on the Toxicology 1 Studies of Cobalt Metal (CAS No. 7440-48-4) in F344/N Rats and B6C3F1/N Mice 2 and Toxicology and Carcinogenesis Studies of Cobalt Metal in F344/NTac Rats and 3 B6C3F1/N Mice (Inhalation Studies). (TR-581). Research Triangle Park, NC.

Dr. Anatoly Zhitkovich

NAS-Identified Expert

Brown University

Comments by **Anatoly Zhitkovich**, PhD
Professor of Medical Science
Brown University

Q. #1: Association between lung and adrenal tumor formation

a) An analysis of the results of NTP inhalation exposure studies in rats found an apparent association between the occurrence of pulmonary non-neoplastic lesions and the development of pheochromocytomas. This plausible association has been attributed to the **adrenal response arising from systemic hypoxemia** due to the reduced gas exchange induced by the lung lesions and the accompanying fibrosis and chronic inflammation.

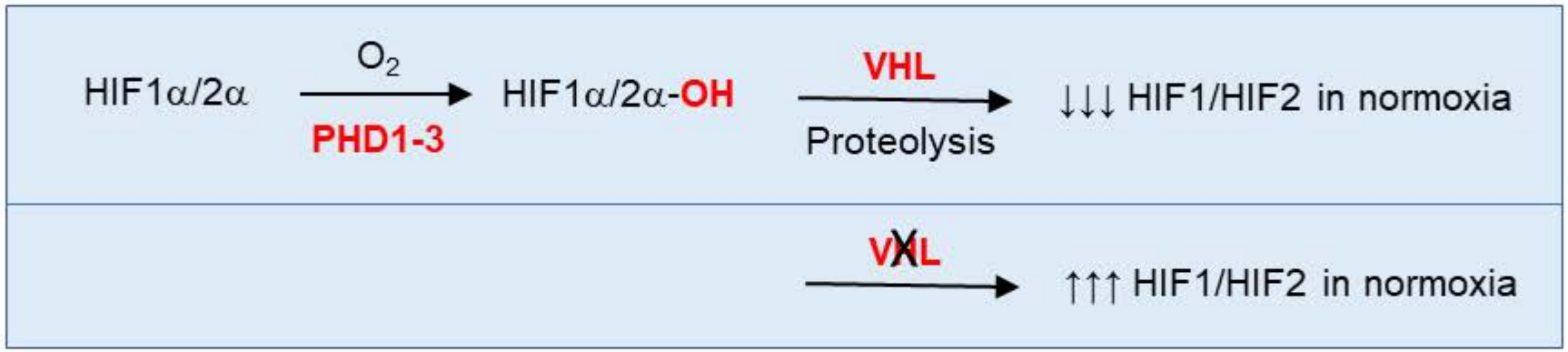
b) Assessment of the dependence of the tumor types impacts upon the method used to estimate composite cancer risk. A combined tumor analysis may not be appropriate if tumors do not form independently. The IRIS program is seeking discussion on a **plausible association between lung and adrenal gland tumors** associated with exposure to cobalt and cobalt compounds.

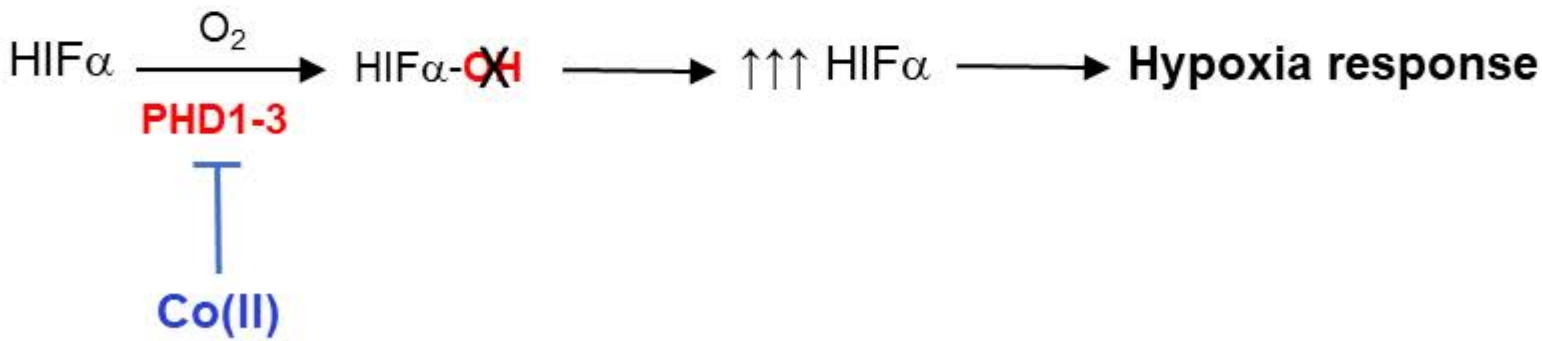
Bullets and emphasis by A.Z.

Plausible tumor formation via direct effects of Co(II) on adrenal cells

Co(II) ions are hypoxia mimetics

Hypoxia response: transcription program by upregulated HIF1α and HIF2α





Comments by Anatoly Zhitkovich, PhD

VHL mutations: von Hippel-Lindau human cancer syndrome

Constitutively active HIFs
von Hippel-Lindau syndrome

- **Pheochromocytoma**
- **Kidney carcinoma**
- **Pancreatic tumors**
- **Hemangioblastomas**

Systemic tumors after inhalation of Cobalt

- **Pheochromocytoma (+/+)**
- Pancreatic tumors (metallic Co, +)
- Kidney tumors (+/-)
- Hemangiosarcomas (Co^{2+}, male mice)

- Systemic distribution of Co: yes
- Adrenal gland accumulation of Co: ?

Comments by Anatoly Zhitkovich, PhD

Q. #1: Association between lung and adrenal tumor formation

a) … adrenal tumors arising from systemic hypoxemia due to lung injury

Basis for this question:

Ozaki K. et al. Association of adrenal pheochromocytoma and lung pathology in inhalation studies with particulate compounds in the male F344 rat - the National Toxicology Program experience. *Toxicol. Pathol.* 2002;30(2):263-270.

Weaknesses in the proposed association of adrenal tumors with hypoxia:

- No evidence that chronic hypoxia causes adrenal tumors
- No measures of hypoxia were used in Ozaki et al. study

Comments by Anatoly Zhitkovich, PhD

Q. #1: Association between lung and adrenal tumor formation

a) … adrenal tumors arising from systemic hypoxemia due to lung injury (Ozaki, 2022)

Weaknesses in the proposed association with lung pathology (fibrosis, inflammation):

- Study was limited to male rats although data for female rats were available
- No association between adrenal medullar hyperplasia and lung pathology was found
- Several chemicals with lung pathology did not increase the number of adrenal tumors

Positive association:

1. Cobalt sulfate
2. Nickel oxide
3. Nickel subsulfide
4. Indium phosphide
5. Talc

No tumors despite lung pathology:

1. Molybdenum trioxide
2. Nickel sulfate
3. Vanadium pentoxide
4. Gallium arsenide (severe pathology)

But:

- Co(II) and Ni(II) are hypoxia mimetics; may act directly on the adrenal cells
- Two dose-dependent responses associate statistically but not necessarily biologically

Comments by Anatoly Zhitkovich, PhD

Q. #1: Association between lung and adrenal tumor formation

b) … plausible association between lung and adrenal gland tumors …

Is there dependence for pheochromocytoma development on the presence of lung tumors?
- *No evidence yet*

Testing plausibility: - **Presence of both tumors in the same animals?**
- Timing of the appearance of both tumors: lung tumors first?
(to excluding high doses of metallic Co: too few rats w/out tumors)

- **Does a majority of lung carcinogens in rats cause adrenal tumors?**

Potential dependence mechanism:

Systemic immunosuppression by lung tumors permitting growth of adrenal tumors

Support: theoretical considerations, no direct or indirect evidence yet

Science Topic 2: Cellular uptake and tissue disposition

- Although cobalt bioavailability and its influence on carcinogenicity are not fully understood, it is known that cellular uptake of free cobalt ion and particles occur via different processes; differences between uptake and distribution of water-soluble and water-insoluble cobalt compounds could lead to differences in pharmacodynamics.
- Mechanistic information regarding cellular uptake and tissue deposition will be updated and may inform dosimetric adjustments and modeling approaches.

Dr. John Wise Sr.

NAS-Identified Expert

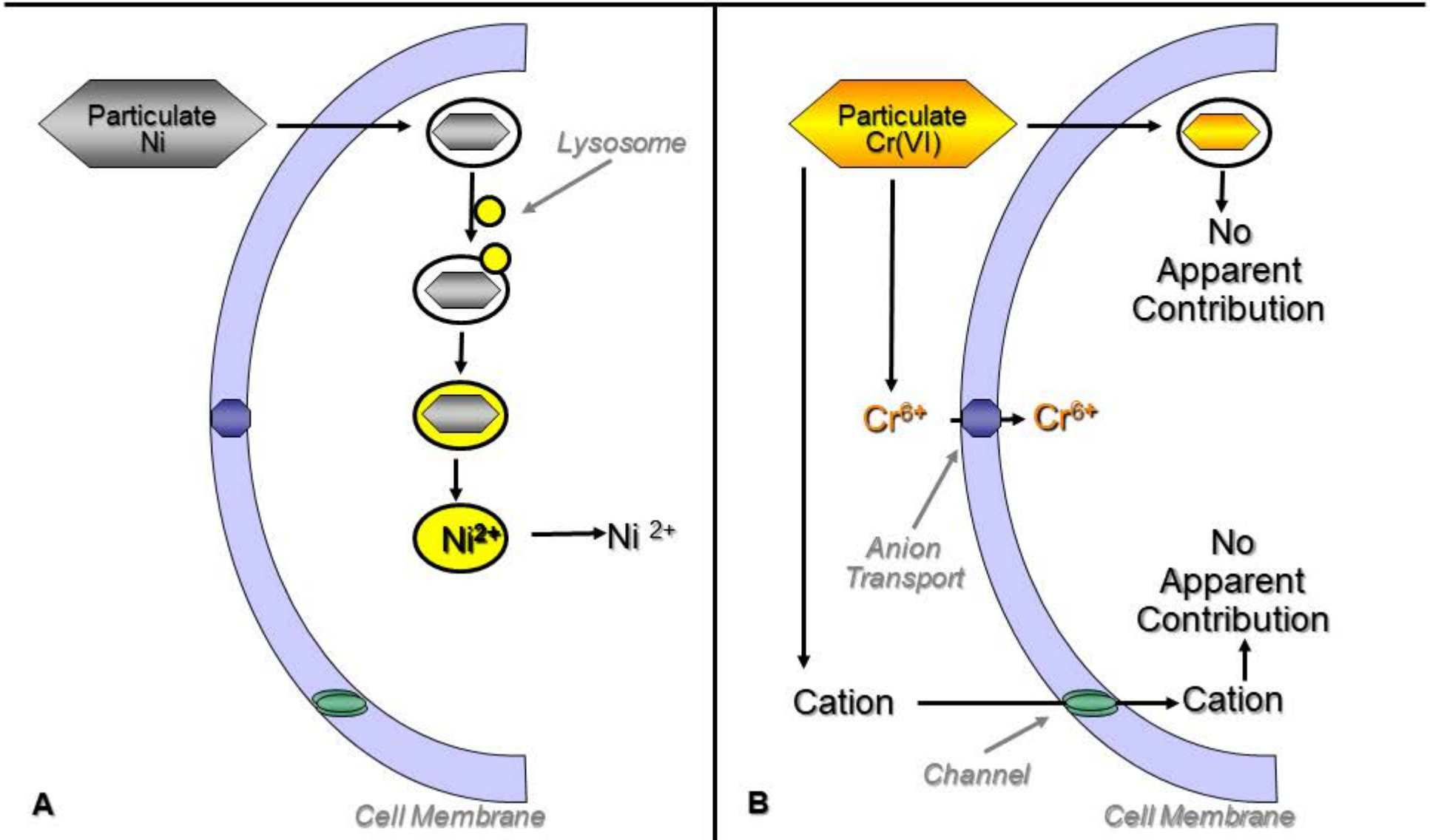
University of Louisville

Topic 2. Cellular Uptake and Tissue Disposition

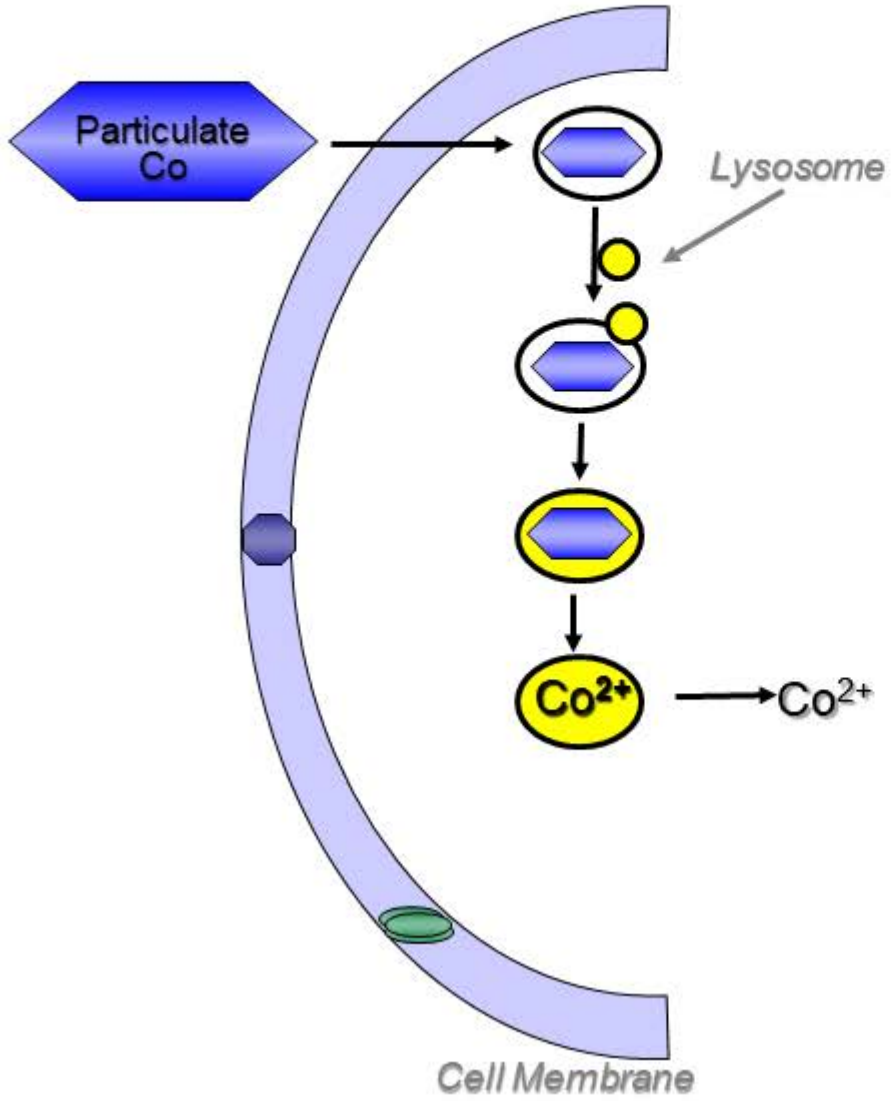
The differences between uptake and intracellular release rates of water-soluble and water-insoluble cobalt compounds could lead to distinct target sites, as well as variations in systemic and intracellular concentrations.

Therefore, mechanistic information regarding cellular uptake and tissue deposition will be updated and may inform selection and application of dosimetric adjustments or modeling approaches.

Physico-Chemical Carcinogenic Mechanism for Known Human Lung Carcinogens



Physico-Chemical Carcinogenic Mechanism for Cobalt Particles



- Key published studies with direct evidence for this mechanism are:
  - Establish particle cell contact required and suggest this mechanism: Smith LJ, Holmes AL, Kandpal SK, Mason MD, Zheng T & Wise JP, Sr. (2014). The cytotoxicity and genotoxicity of soluble and particulate cobalt in human lung fibroblast cells. Toxicol Appl Pharmacol 278(3): 259-65.
  - Establish lysosomal dissolution: Ortega R, Bresson C, Darolles C, Gautier C, Roudeau S, Perrin, L, Janin M, Floriani M, Aloin V, Asuncion C, & Véronique 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
  - Several additional studies support increased Co after particulate exposure and are cited in reviews in assessment

Topic 2 Comments and Considerations

- The IRIS Assessment Plan and Protocol for Assessing Cancer Risk from Inhalation Exposure to Cobalt and Cobalt Compounds is on point for this issue and has it described accurately
- The plan to update the mechanistic information regarding cellular uptake and tissue deposition with the expectation that it may inform selection and application of dosimetric adjustments or modeling approaches is appropriate
- For precedent informing dosimetric adjustments and modeling approaches, consider the assessments for nickel as it has the same underlying mechanism and issue with particulate versus soluble compounds
  - Nickel may have been done too long ago for these adjustments
- If prior nickel assessments avoid the issue, EPA could consider reviewing the primary literature to try and develop an adjustment factor to quantify the difference in magnitude of the rate of uptake and tissue deposition for particles versus soluble to apply in dosimetric adjustments and modeling approaches
- IRIS Assessment Plan and Protocol Cites: U.S. EPA (2008); Lison et al. (2018); NTP (2016); NTP (2021); OEHHA (2020).
  - Suggest dropping U.S. EPA (2008); Lison et al. (2018) as not the right fit

Science Topic 3: Cobalt Particle Toxicity

- In addition to potential differences in particle ion uptake and distribution that might influence tissue dosimetry, cobalt is a redox-active transition metal. Cobalt particles may have a greater effect than ions in catalyzing production of reactive oxygen species (ROS). How cobalt ions are released in vivo also differs between water-soluble and water-insoluble cobalt compounds. Updating the mechanistic evidence concerning whether cobalt particles may elicit direct toxicity contributing to carcinogenesis will help inform the choice of the particle lung dose metric used for rodent-to-human extrapolation and dose-response.

Dr. John Wise Sr.

NAS-Identified Expert

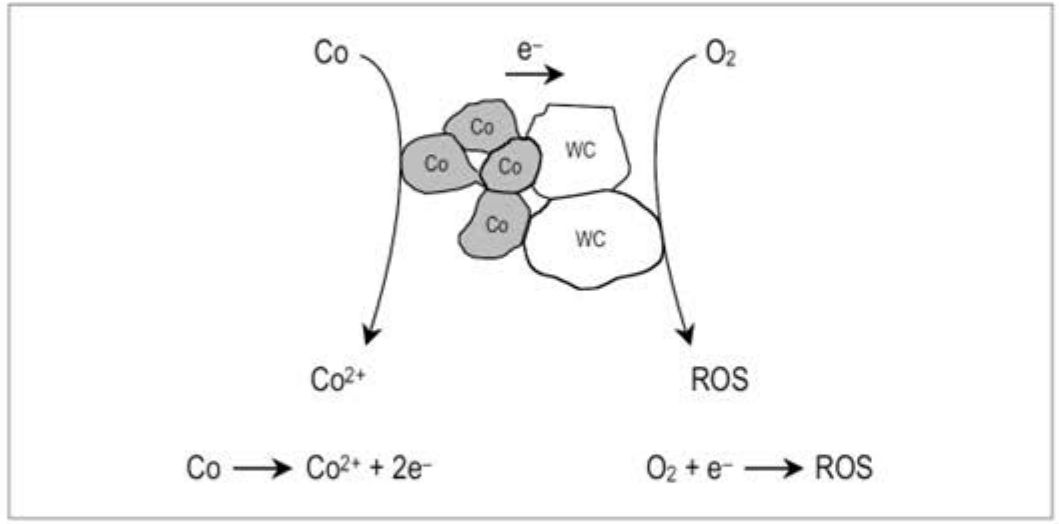
University of Louisville

Topic 3. Cobalt Particle Toxicity

Updating the mechanistic evidence concerning whether cobalt particles may elicit direct toxicity contributing to carcinogenesis will help inform the choice of the particle lung dose metric used for rodent-to-human extrapolation and dose-response.

Physico-Chemical Mechanism for Increased Toxicity of Cobalt Particles

Figure 2. Mechanism proposed for release of reactive oxygen species (ROS) from buffered aqueous suspensions of cobalt/tungsten carbide (Co/WC) mixtures (hard metals)

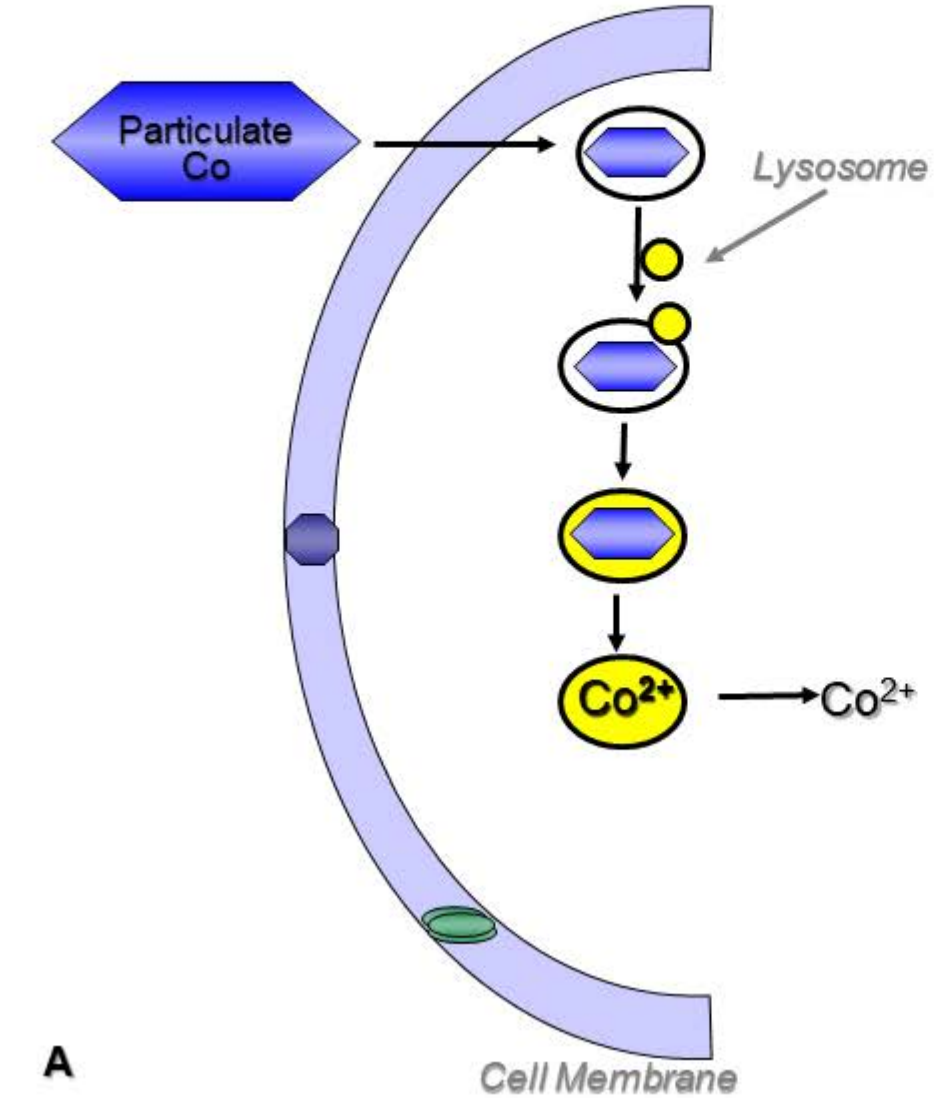


Adapted from Zanetti & Fubini (1997)
Cobalt is progressively oxidized and solubilized; oxygen is activated at the carbide surface.
e⁻, electron

Figure from: (International Agency for Research on Cancer). (2006). Cobalt in hard metals and cobalt sulfate, gallium arsenide, indium phosphide and vanadium pentoxide. Lyon, France. https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Cobalt-In-Hard-Metals-And-Cobalt-Sulfate-Gallium-Arsenide-Indium-Phosphide-And-Vanadium-Pentoxide-2006.

- References cited [IARC (2006); NTP (2016)] seem to limit this mechanism to cobalt-tungsten particles
  - NTP 2016 appears to only refer to cobalt-tungsten for this mechanism
  - IARC 2006 does state: "Cobalt-metal particles produce mutagenic effects in vitro by two different mechanisms: directly through the production of ROS resulting in DNA damage…" but there is no citation and no evidence for this comment provided
  - Moreover, IARC 2006 goes on to state: "*Cobalt-metal particles are weak inducers of reactive oxygen species in vitro, but this effect is greatly enhanced by the presence of tungsten carbide particles.*"

Reminder - The Physico-Chemical Carcinogenic Mechanism for Cobalt Particles May Inform



- A mechanism of cobalt particle toxicity being due to ROS and not cobalt ions appears to be inconsistent with the literature establishing this intracellular dissolution mechanism
- Need to reconcile the concept of cobalt particles being toxic due to ROS and not due to cobalt ions with the literature that show cobalt ions from the particles are the concern
- May need to focus on primary literature to pursue this particular concept

Topic 3 Comments and Considerations

- The suggestion that cobalt particles alone may have a greater effect than cobalt ions in catalyzing production of reactive oxygen species (ROS) appears to be speculative
- Cobalt-tungsten particles may exhibit a greater effect than either cobalt ions or cobalt particles in catalyzing production of reactive oxygen species (ROS)
- The IRIS Assessment Plan and Protocol for Assessing Cancer Risk from Inhalation Exposure to Cobalt and Cobalt Compounds needs to clarify whether, for this specific aspect, it is drawing this consideration from data from cobalt-tungsten particles and considering cobalt-tungsten particles to be indicative of all cobalt particles.
  - If yes, such a conclusion may be inconsistent with the toxicology data for cobalt-tungsten particles
  - If no, careful inspection of the secondary and primary literature is needed
- The plan to update the mechanistic evidence concerning whether cobalt particles, themselves, may elicit direct toxicity contributing to carcinogenesis to help inform the choice of the particle lung dose metric used for rodent-to-human extrapolation and dose-response may only be applicable to the special case of cobalt-tungsten particles as only these particles have been shown to produce significant amounts of ROS – thus, this aspect of the plan may need revision

Science Topic 4: Proposed MOA of cobalt carcinogenicity

- There is evidence that cobalt-induced neoplastic development likely involves pathways of genotoxicity, oxidative stress (and generation/scavenging of ROS), and stabilization of hypoxia-inducible factor 1α. Other evidence suggests that cobalt genotoxicity involves primarily clastogenic effects, as well as direct and indirect DNA damage and inhibition of DNA repair. Updating the current evidence in the proposed cobalt cancer MOA, including capturing any new evidence of mechanistic responses beyond those previously described, will help inform the dose-response analyses, pharmacokinetic evaluations, and animal-to-human extrapolation methodologies.
- Substances that can release cobalt ions in vivo, both water soluble and insoluble, likely define the domain of applicability for this assessment.

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Topic 4. Proposed MOA of Cobalt Carcinogenicity

Updating the current evidence in the proposed cobalt cancer MOA, including capturing any new evidence of mechanistic responses beyond those previously described, will help inform the dose-response analyses, pharmacokinetic evaluations, and animal-to-human extrapolation methodologies.

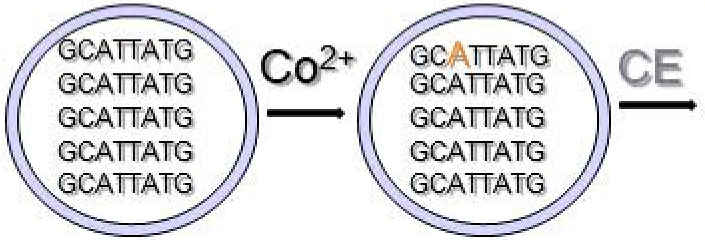
Topic 4 Comments and Considerations

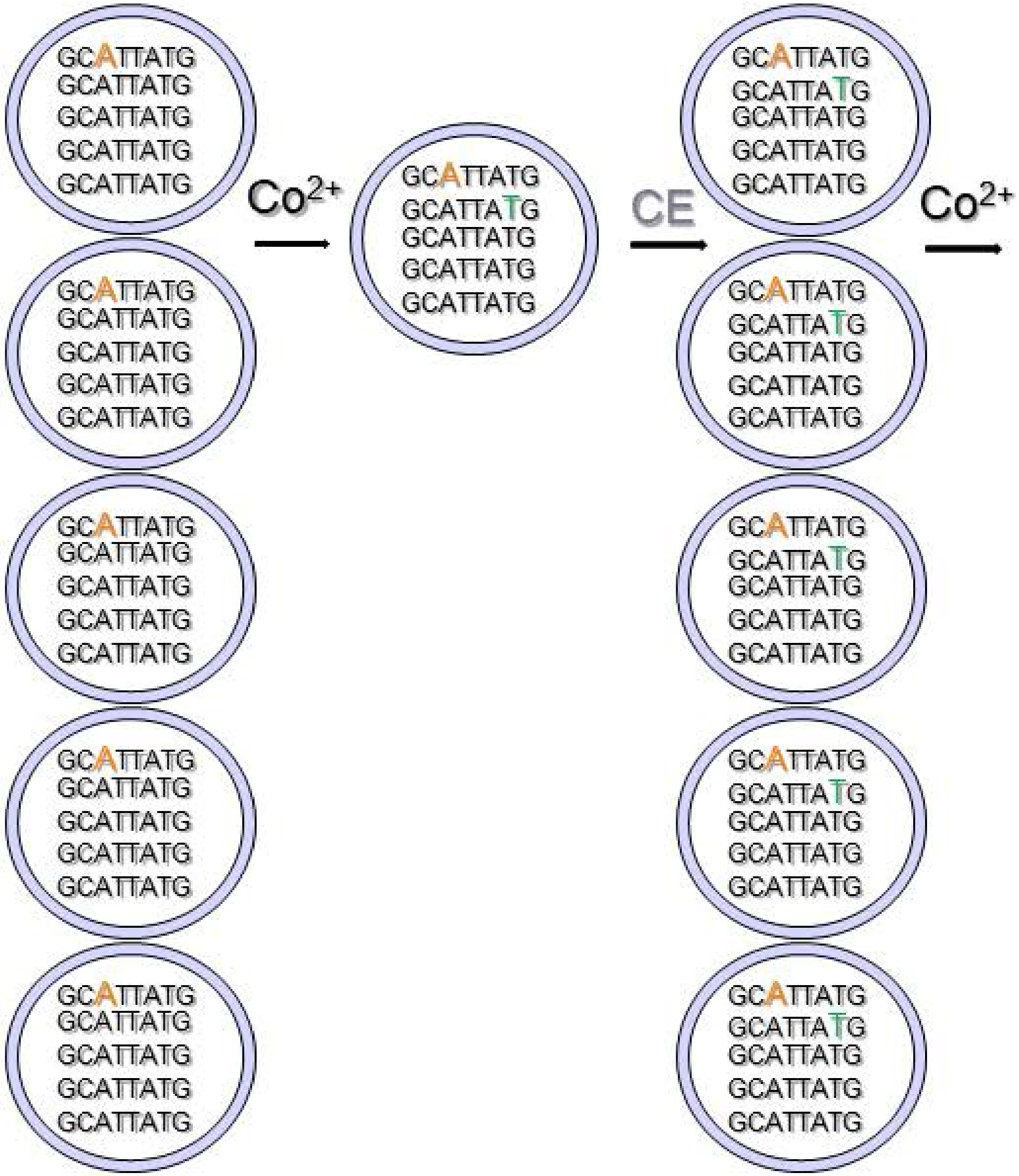
From the IRIS Assessment Plan and Protocol for Assessing Cancer Risk from Inhalation Exposure to Cobalt and Cobalt Compounds:

- There is evidence that cobalt-induced neoplastic development likely involves:
  - Pathways of genotoxicity
  - Pathways of oxidative stress
  - Pathways of stabilization of hypoxia-inducible factor 1α
  - Cobalt genotoxicity is primarily clastogenic effects, direct & indirect DNA damage & inhibition of DNA repair
- Previous assessments have found the evidence generally inconsistent on whether inhaled cobalt carcinogenicity involves a mutagenic MOA

- Care should be taken when evaluating whether or not to use a mutagenic MOA
  - Data certainly implicate cobalt as a clastogen
  - Tendency to confuse words "mutagenic" as causing mutations vs. "clastogenic" as not causing mutations - but toxicologically clastogens are also mutagens and chemicals that are genotoxic are also mutagens
  - Typically, the distinct point with clastogens is the type of damage i.e. they may not cause simple base substitution mutations and instead break strands of DNA, causing larger scale mutagenic events

Clonal Expansion of Small Mutations – Unlikely Mutagenic Mechanism for Cobalt Continued



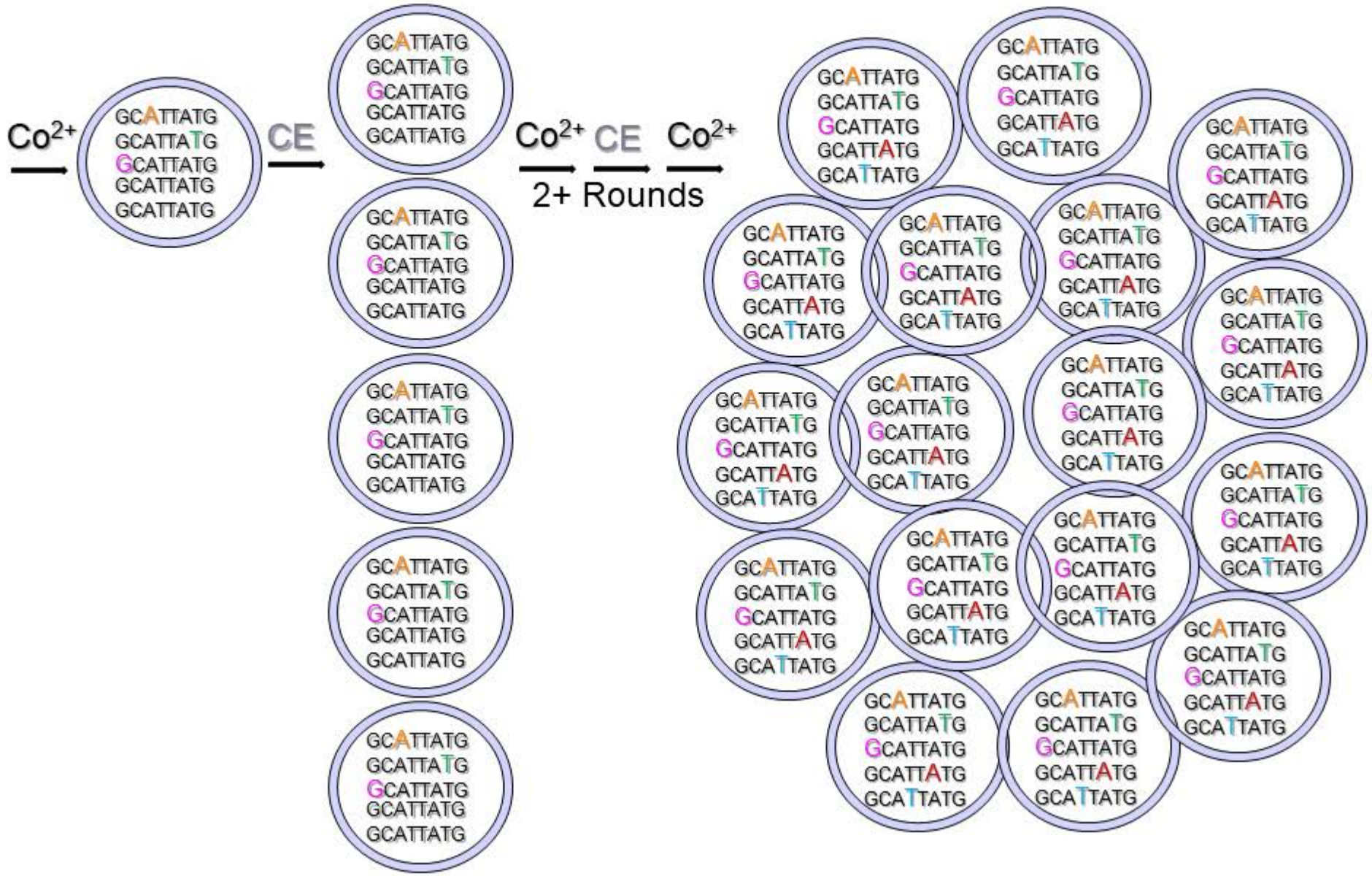


The DNA sequences and the specific base changes are random and fictional for the purposes of illustration. They are not related to any specific genes or mutations. Each line is meant to represent a difference gene.

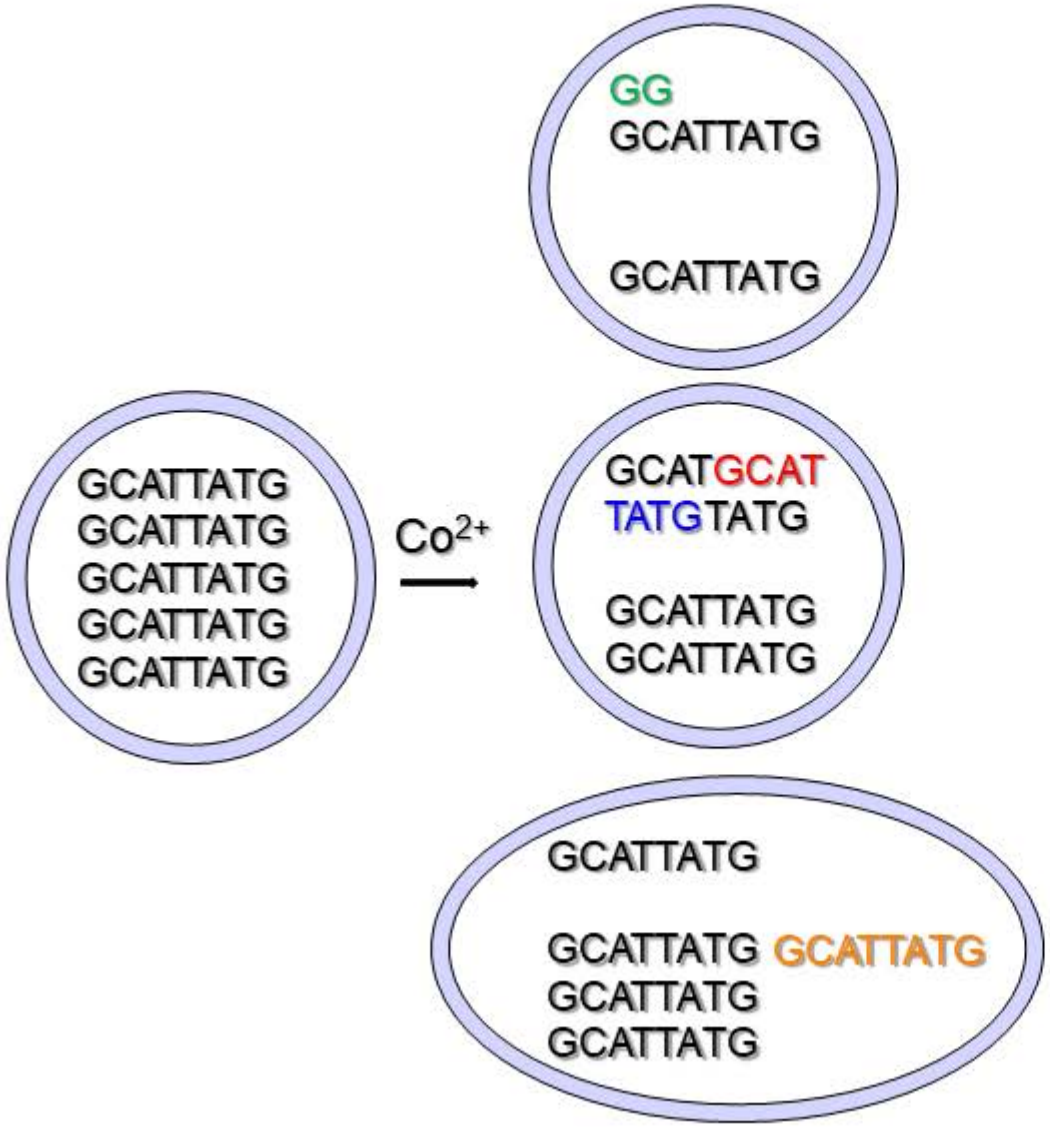
Co^{2+} = Cobalt

CE = Clonal Expansion

Clonal Expansion of Small Mutations – Unlikely Mutagenic Mechanism for Cobalt Continued



Clastogenic-Type Mechanism Leads to Large Mutagenic DNA Sequence Alterations



Oversimplification of possible clastogenic type outcomes meant to illustrate mutagenic potential of clastogens. Outcomes shown do not reflect the full spectrum of possible outcomes nor the magnitude of possible outcomes.

The DNA sequences and the specific base changes are random and fictional for the purposes of illustration. They are not related to any specific genes or mutations. Each line is meant to represent a difference gene.

Co^{2+} = Cobalt

CE = Clonal Expansion

Topic 4 Comments and Considerations

- The IRIS Assessment Plan and Protocol for Assessing Cancer Risk from Inhalation Exposure to Cobalt and Cobalt Compounds is on point for this issue and has it described accurately

- The plan to update the current evidence in the proposed cobalt cancer MOA, including capturing any new evidence of mechanistic responses beyond those previously described, will help inform the dose-response analyses, pharmacokinetic evaluations, and animal-to-human extrapolation methodologies is appropriate

- Care should be taken when evaluating whether or not to use a mutagenic MOA
  - Data certainly implicate cobalt as a clastogen
  - Tendency to confuse words "mutagenic" as causing mutations vs. "clastogenic" as not causing mutations - but toxicologically clastogens are also mutagens and chemicals that are genotoxic are also mutagens
  - Typically, the distinct point with clastogens is the type of damage i.e. they may not cause simple base substitution mutations and instead break strands of DNA, causing larger scale mutagenic events

Dr. Anatoly Zhitkovich

NAS-Identified Expert

Brown University

Comments by **Anatoly Zhitkovich**, PhD
Professor of Medical Science
Brown University

Q. #4: Proposed MOA of cobalt carcinogenicity

While not fully understood, there is evidence that cobalt-induced neoplastic development likely involves pathways of

- Genotoxicity
- Oxidative stress (and generation/scavenging of ROS), and
- Stabilization of hypoxia-inducible factor 1α (HIF-1α).

Evidence with differing water-insoluble and water-soluble cobalt compounds suggests cobalt genotoxicity involves primarily clastogenic effects, as well as direct and indirect DNA damage and inhibition of DNA repair.

Previous assessments have found the evidence generally inconsistent on whether inhaled cobalt carcinogenicity involves a mutagenic MOA, and do not apply age-dependent adjustment factors (ADAFs) in unit risk estimates.

Bullets and emphasis by A.Z.

Q. #4: Proposed MOA of cobalt carcinogenicity:

via Stabilization of hypoxia-inducible factor 1α (HIF-1α)?

Reasons to suspect involvement of HIF1 and/or HIF2 in cobalt carcinogenicity:

- Co(II) ions are hypoxia mimetics (HIF1/2 inducers)
- Elevated hypoxia gene expression signature in more aggressive tumors
- Constitutive activation of the hypoxia response → von Hippel-Lindau cancer syndrome

HIF1 and/or HIF2 in cobalt carcinogenicity? **More likely HIF2 for systemic tumors**

- Importance of HIF2 in VHL loss-induced cancers (mechanistic studies, success of Belzutifan)
- Loss of HIF1α expression in the majority of VHL-induced kidney cancers: tumor suppressor?
- HIF1 activation inhibits growth of normal cells (Co-treated lung cells: Luczak MW, 2021)
- HIF2 in the development of lung tumors: plausible but not proven
- Lung tumors: highly oxygenated tissue, HIF1/2 may promote growth of advanced tumors

Comments by Anatoly Zhitkovich, PhD

Q. #4: Proposed MOA of cobalt carcinogenicity: **Oxidative Stress**

Potential causes of oxidative stress by Cobalt:

- Direct redox activity of Co(II) – Fenton-like reactions (established mechanism)
- Co(II) binding and inhibition of antioxidant proteins (plausible, evidence-?)
- Stimulation of ROS production by cells (NOX4 in rat lungs, Ton TT 2021)
- Inflammation

In vitro (cellular) studies: Consistent data on increased oxidative stress

- Ascorbate depletion (Salnikow KS, 2004)
- Oxidation of redox-sensitive probes (Patel E, 2012; Kirkland D, 2015; Ton TT, 2021)
- 8-oxo-dG formation – Comet assay with OGG1 treatment (Kirkland D, 2015)

In vivo studies in rats:

- Increased oxidative DNA damage in kidney, lung and liver – i.p. Co(II) (Kasprzak KS, 1994)
- No clastogenic damage in bone marrow by oral Co(II) (Kirkland D, 2015) – bioavailability?
- Increased 8-oxo-dG in rat lung after inhalation of Co metal dust (Ton TT, 2021)

Comments by Anatoly Zhitkovich, PhD

Q. #4: Proposed MOA of cobalt carcinogenicity: **Genotoxicity**

In vitro (cells in culture) genotoxicity: Generally positive findings

- 8-oxo-dG formation – Comet assay with OGG1 treatment (Kirkland D, 2015)
- DNA ss-strand breaks (Kirkland D, 2015; others)
- Chromosomal damage (micronuclei, chromatid breaks and gaps; Smith LJ 2014, others)
- Sister chromatid exchanges (Hartwig A, 1991)

In vivo studies in rats: Positive findings for rat lungs

- Oxidative DNA damage in kidney, lung and liver – i.p. Co(II) (Kasprzak KS, 1994)
- No clastogenic damage in bone marrow after oral exposure (Kirkland D, 2015)
- 8-oxo-dG in rat lung after inhalation of Co metal dust (Ton TT, 2021)

Proposed mechanisms of genotoxicity:

- **Oxidative DNA damage by Co(II)-induced ROS/oxidative stress**
- Inhibition of DNA repair (UV), but no effect on mutagenesis and clastogenesis of γ-radiation

Comments by Anatoly Zhitkovich, PhD

Q. #4: Proposed MOA of cobalt carcinogenicity: **Mutagenicity**

Bacterial/Ames mutagenicity tests: *detection of point mutations, not deletions*

- Positive results (NTP studies)
- Negative findings (Kirkland 2015; others)

Mammalian tests: *detection of point mutations and to a lesser degree, deletions*

- Positive at *Hprt* locus in hamster V79 fibroblasts (20 h Co^{2+} ions; Hartwig A. 1991)
- Positive at *Hprt* locus in mouse lymphoma cells (24 h metallic Co; Kirkland D, 2015)
- Positive at *gpt* locus in G12-V79 transgenic line (24 h Co; Kitahara J, 1996)
- Negative at *Tk* and *HPRT* loci in mouse lymphoma cells (3 h Co; Kirkland D, 2015)
- Small *Tk*$^-$ colonies in Co-treated cells, indicative of large deletions (Kirkland D, 2015)

Insensitivity of bacterial and mammalian test systems for detection of:

- Chromothripsis (caused by micronuclei; important in lung and pancreatic cancers)
- Dinucleotide repeat-targeted mutations (genes contained only trinucleotide repeats)

Comments by Anatoly Zhitkovich, PhD

Q. #4: Proposed MOA of cobalt carcinogenicity: **Mutagenicity**

Riva L. et al. The mutational signature profile of known and suspected human carcinogens in mice. *Nature Genet.* 2020;52(11):1189-1197.

Cobalt metal-induced mouse lung tumors: *whole genome sequencing results*

- Higher number of single nucleotide mutations in comparison to spontaneous lung tumors
- Higher number of dinucleotide substitutions vs other chemicals (n=8) and spontaneous
- Specific dinucleotide mutation signature (mID8) was detected
- Higher frequency of *Kras* mutations than in lung tumors induced by other chemicals (n=8)

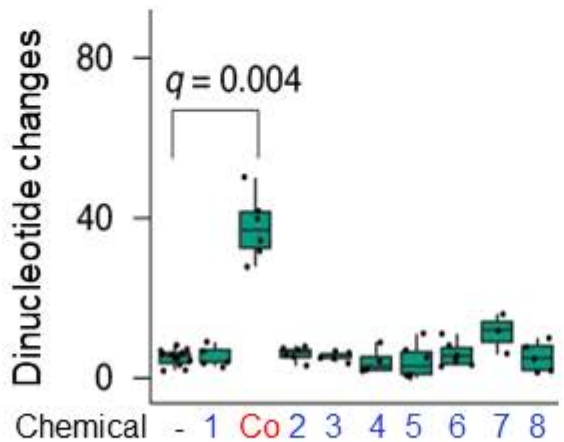


Fig. 3A (cropped). Riva L, 2020.
Mouse lung tumors arising spontaneously (-) and induced by various chemicals

Comments by Anatoly Zhitkovich, PhD

Q. #4: Proposed MOA of cobalt carcinogenicity:

"Previous assessments have found the evidence generally inconsistent on whether inhaled cobalt carcinogenicity involves a mutagenic MOA".

Cobalt carcinogenicity is consistent with a mutagenic MOA.

Cobalt is a a genotoxic mutagen:

- Induces premutagenic lesions (DNA breaks, 8-oxo-dG)
- Chromosomal mutations (aberrations, chromatid gaps)
- Causes micronuclei – precursors of chromothripsis (massive chromosomal rearrangements)
- Mutagen in mammalian cells

Cobalt mutagenesis in mouse lung tumors:

- Higher mutational load than spontaneous lung tumors
- Very high frequency of dinucleotide mutations (poorly detectable by standard tests)
- Unique dinucleotide mutation signature (excludes endogenous processes)

Public Commenters

Dr. Vanessa Viegas

Public Commenter

Cobalt Institute

January 11th 2023

US EPA IRIS assessment on Co and Co compounds:

Science Webinar

Cobalt Institute Public Comments

COBALT INSTITUTE

For a comprehensive response on the points raised in the presentation today, please refer to documents in the relevant public docket:

"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 attachment:

"Attachment 1. Stantec ChemRisk Cobalt Comments IRIS"

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COBALT AND COBALT SUBSTANCES

At least two groups of cobalt substances exist

- Supported by: existing data, new testing strategy, IARC opinion

Mutagenicity exclusion in Co MOA for carcinogenicity

- Extensive genotoxicity database
- OECD CoCAM conclusion

Data generation in next 3 – 5 years

- MOA, toxicokinetic and sub-chronic data – inform on poorly soluble substances
- 'Site of contact' in vivo genotoxicity and inflammation data – inform on threshold, genotoxicity and inflammation
- Oral carcinogenicity study – inform on relevant local and systemic findings

Human epidemiology data should be used in a WoE

- Large, recent studies show lack of increase of cancer in workplace
- Series of papers – First adverse effect in respiratory tract linked to reduction in lung function ('workplace asthma') – with threshold

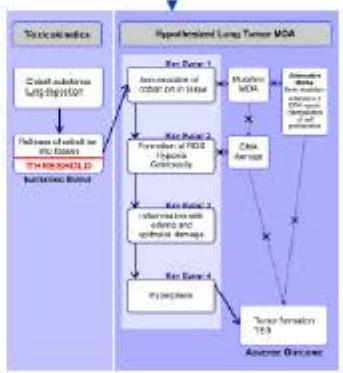
MOA = mode-of-action; **WoE** = weight of evidence

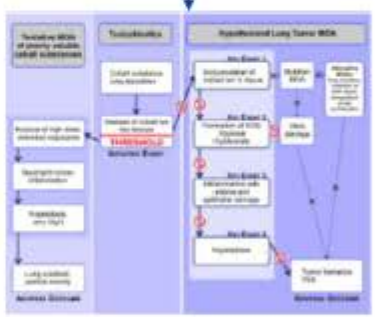
Reactive substances
(e.g. Co sulfate, Co metal)

- **Co metal** → highly soluble in all biologically relevant fluids
- Supported by recent IARC conclusions

Poorly reactive / poorly soluble substances
(e.g. tricobalt tetraoxide)

- **Tricobalt tetraoxide** → poorly soluble in all biologically relevant fluids
- Supported by recent IARC conclusions





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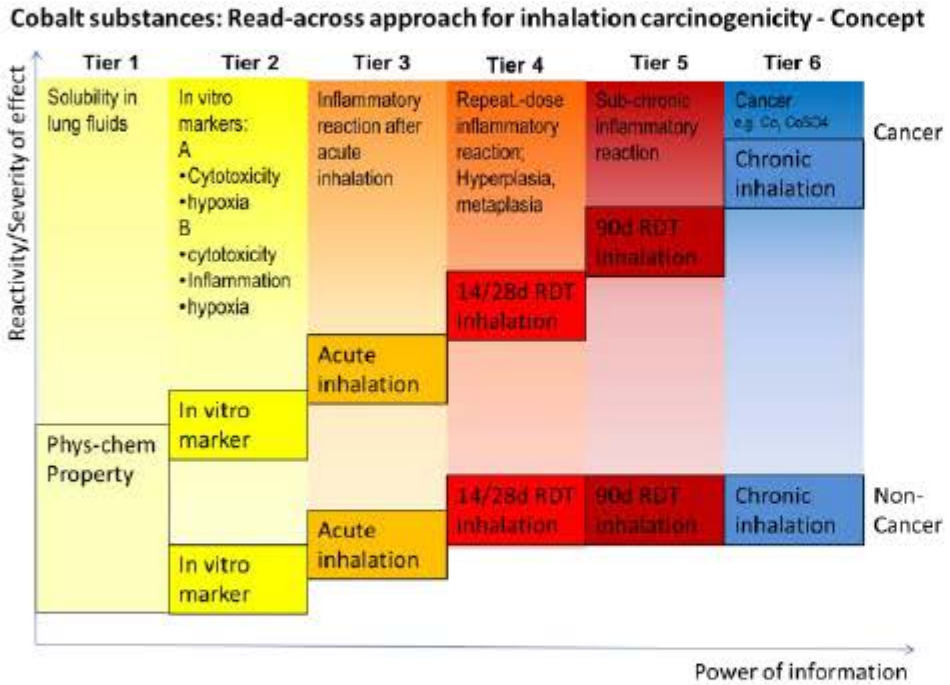
COBALT AND COBALT COMPOUNDS: MODE OF ACTION BASED TIERED APPROACH



- **Read-across and grouping approach published in series of papers in Regulatory Toxicology and Pharmacology (2022)**
  - Uses MOA data to predict longer-term toxicity of Co substances
- **High quality in vitro and in vivo data show MOA for carcinogenicity**
  - Oxidative stress, cytotoxicity, hypoxia and a sustained inflammatory response – threshold events
- **Mutagenicity excluded as MOA for Co carcinogenicity**
- **IARC 2022 conclusions support different groups**
  - Carcinogenicity classifications for reactive cobalt substances and lack of classification for poorly reactive substances.
- **Upcoming data generation**
  - 90-day RDT inhalation study – support MOA poorly soluble group
  - In vivo genotoxicity – support threshold, MOA proposed

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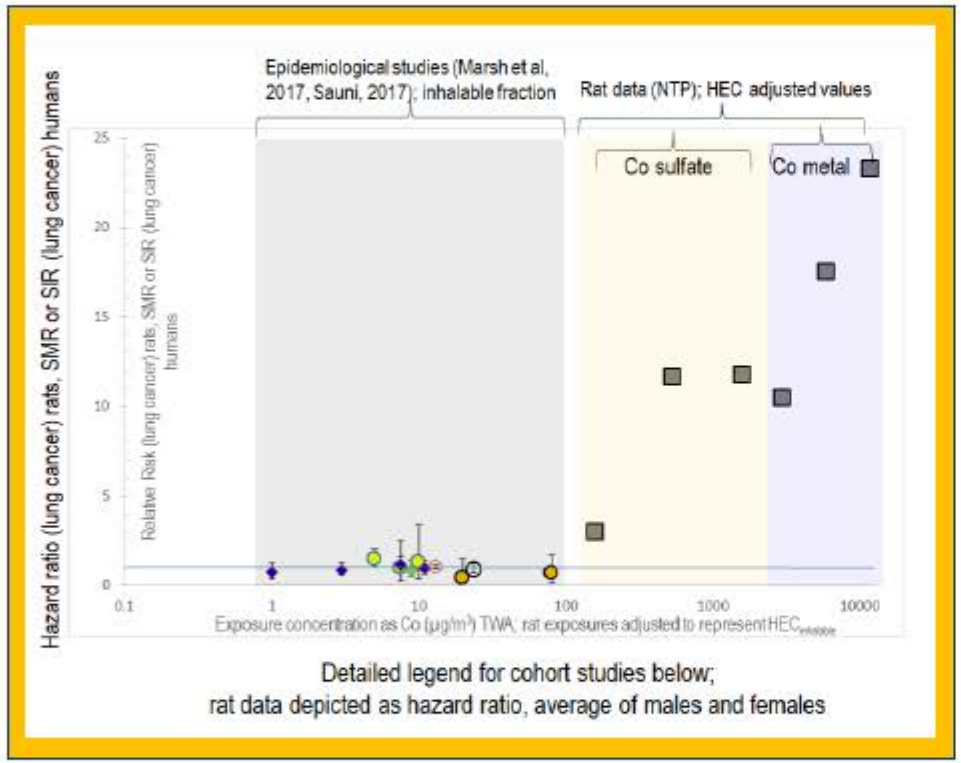
COBALT AND COBALT COMPOUNDS: DOSE-RESPONSE FOR CARCINOGENICITY



- **Exclusion of direct genotoxicity as a MOA for cobalt-induced carcinogenicity**
- **Recent, high quality, large epidemiology study in hard metal industry and study in cobalt-only industry**
  - No increased risk of cobalt-induced cancer at exposures observed
  - Do not support a linear extrapolation at low doses
  - Do not support a high potency for cancer
- **Weight-of-evidence approach (reactive substances)**
  - Human data (negative for cancer) can inform on carcinogenic risk at low-end of dose-response, when using the NTP rodent carcinogenicity data (positive for cancer) for quantitative analysis
- **Scientific concerns**
  - Layers of conservative assumptions inherent within a linear extrapolation at low doses in the inhalation unit risk estimate

HEC = human equivalent concentration; **SMR** = standardised mortality ratio and **SIR** = standardised incidence ratio; **WoE** = weight of evidence

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COBALT AND COBALT COMPOUNDS: INDEPENDENCE OF TUMOURS (NTP STUDIES)

Pheochromocytomas

- Predominant systemic finding in Co inhalation studies
- Well-established response to respiratory distress and hypoxia
- Statistical analysis of 9 NTP carc inhalation studies (range of lung effects and association with pheochromocytoma): Concluded an overall association between lung impairment *by any cause* and an elevated incidence of adrenal pheochromocytoma in NTP inhalation studies.

Mononuclear cell leukemia
Kidney
Pancreatic islets

- Lack a dose-response
- Occur in only one sex (either Females or Males) in rats
- Lack of historical control database for F344/NTac rats
- F344/NTac discontinuation after 1 inhalation study (Co metal)

These aspects cast doubt on the interpretation that the individual systemic tumors are independent and directly related to cobalt

Systemic findings will be investigated in an oral carcinogenicity study with a bioavailable Co substance

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Thank you!