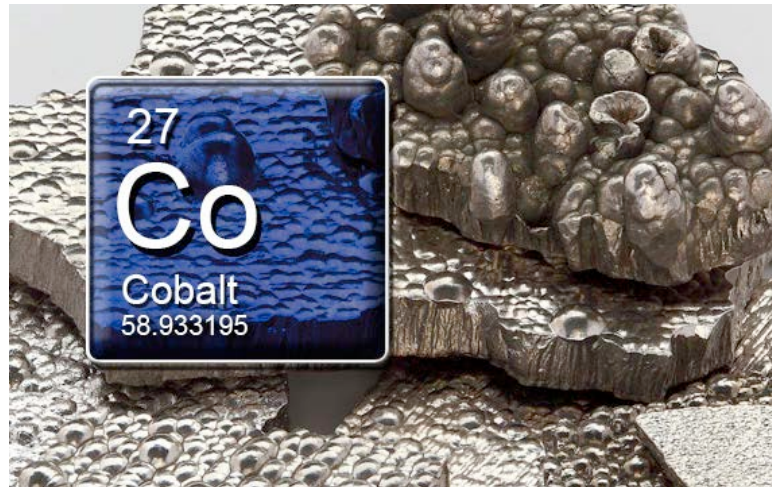


# Studies in Experimental Animals



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## Outline

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Overview of studies in experimental animals

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Study quality assessment

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Cancer assessment

- Inhalation tumors in lung and other sites
  - Injection site tumors
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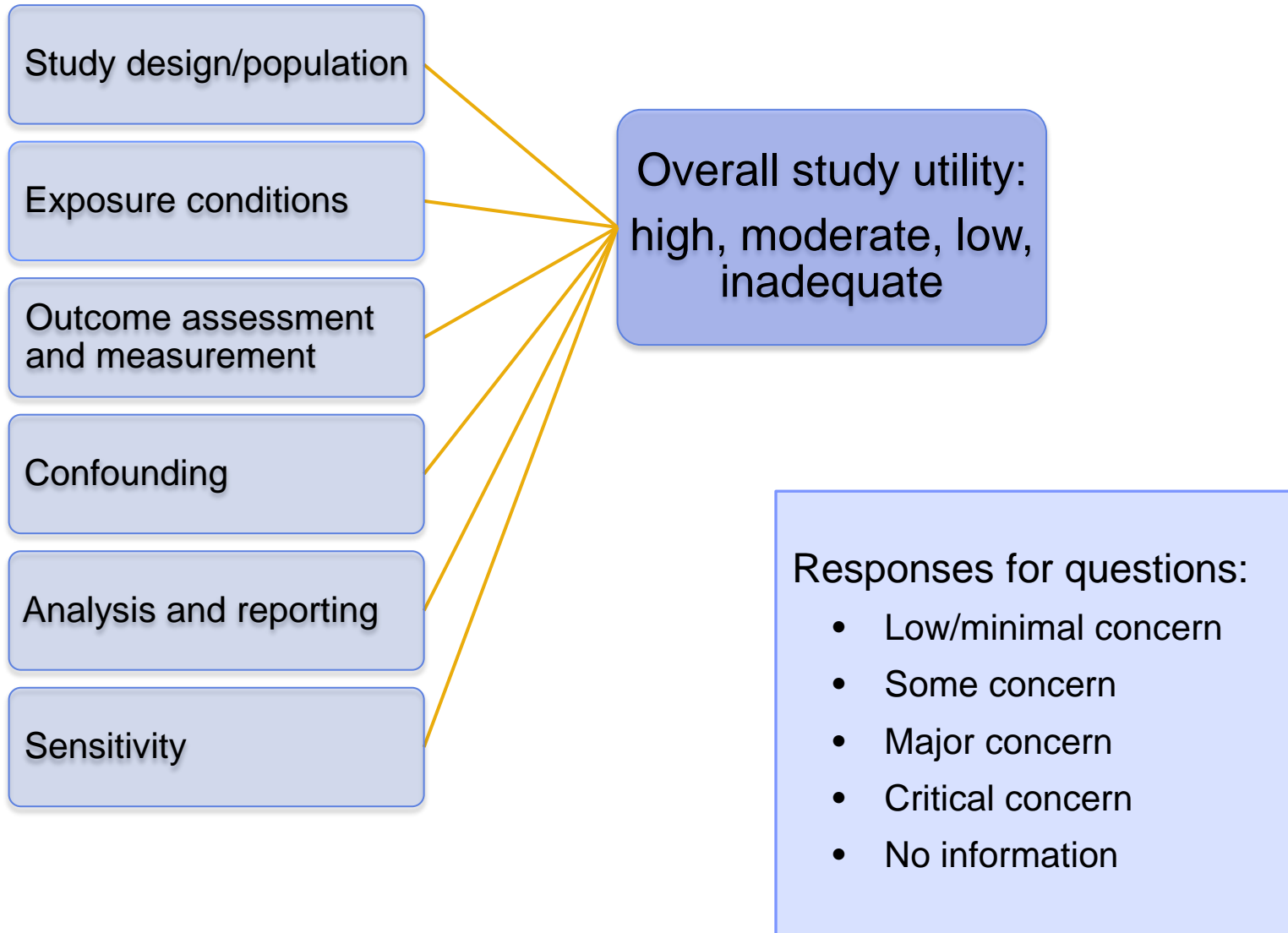
# Carcinogenicity Studies

Cobalt form (# studies)	Species	Route of exposure	Reference (10 pub.)
Water soluble			
Cobalt sulfate heptahydrate (2)	Rat ♂♀ Mouse ♂♀	Inhalation Inhalation	NTP 1998 NTP 1998
Cobalt chloride (1)	Rat ♂	Injection – SC	Shabaan <i>et al.</i> 1977
Cobalt metal (6)	Rat ♂♀ Mouse ♂♀ Rat ♀ Rat ♂♀ Rat ♀ Rat ♂	Inhalation Inhalation Injection – IR Injection – IM Injection – IT Injection – SC	NTP 2014 NTP 2014 Jasmin & Riopelle 1976 Heath 1956 Heath & Daniel 1962 Hansen <i>et al.</i> 2006
Cobalt nanoparticles (1)	Rat ♂	Injection – IM	Hansen <i>et al.</i> 2006
Poorly water soluble			
Cobalt(II) oxide (6)	Hamster ♂ Rat ♂♀ Rat ♂♀ Rat ♂ Rat ♂♀ Mouse ♀	Inhalation Intratracheal instillation Injection – IP Injection – SC Injection – IM Injection – IM	Wehner <i>et al.</i> 1977 Steinhoff & Mohr 1991 Steinhoff & Mohr 1991 Steinhoff & Mohr 1991 Gilman & Ruckerbauer 1962 Gilman & Ruckerbauer 1962
Cobalt sulfide (1)	Rat ♀	Injection – IR	Jasmin & Riopelle 1976

IM = intramuscular, IP = intraperitoneal, IR = intrarenal, IT = intrathoracic, SC = subcutaneous.



# Carcinogenicity Study Quality Assessment





# Carcinogenicity Study Quality Assessment

## Overall study utility

Overall Utility	Studies
High	NTP 1998 (rat and mouse) NTP 2014 (rat and mouse)
Moderate	Hansen <i>et al.</i> 2006 Steinhoff & Mohr 1991 (3 routes) Wehner <i>et al.</i> 1977
Low	Gilman & Ruckerbauer 1962 (rat and mouse) Heath 1956 Heath & Daniel 1962 Jasmin & Riopelle 1976 Shabaan <i>et al.</i> 1977

- All carcinogenicity studies were considered to have some utility for cancer hazard evaluation
- High utility: NTP studies were most informative
  - Sufficient number of animals
  - Both sexes
  - Lifetime treatment
  - Three dose levels
  - Untreated controls
- In general, major limitation in studies with low or moderate utility was low sensitivity
  - Due to, e.g., short duration, single dose, few animals
  - Little concern that duration or single dose would decrease confidence in a positive finding

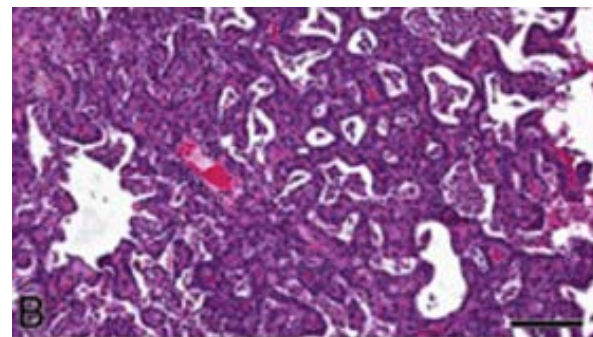
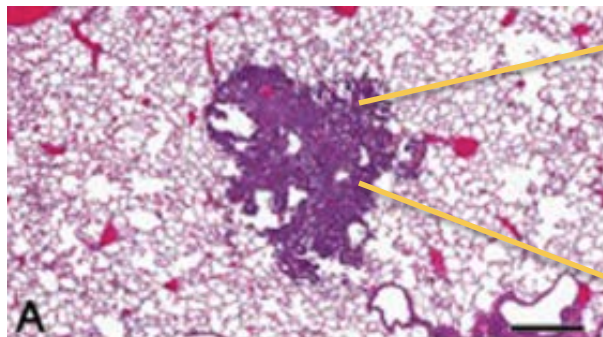


# Inhalation/intratracheal Exposure

## Different types of cobalt compounds caused lung neoplasms after exposure in rodents

Cobalt form	RAT		MOUSE	
	Male	Female	Male	Female
Water soluble ( $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ )	✓	✓	✓	✓
Metal	✓	✓	✓	✓
Poorly water soluble ( $\text{CoO}$ )	✓	✓	NT	NT

Sources: NTP 1998, 2014; Steinhoff and Mohr 1991.  
NT = not tested.

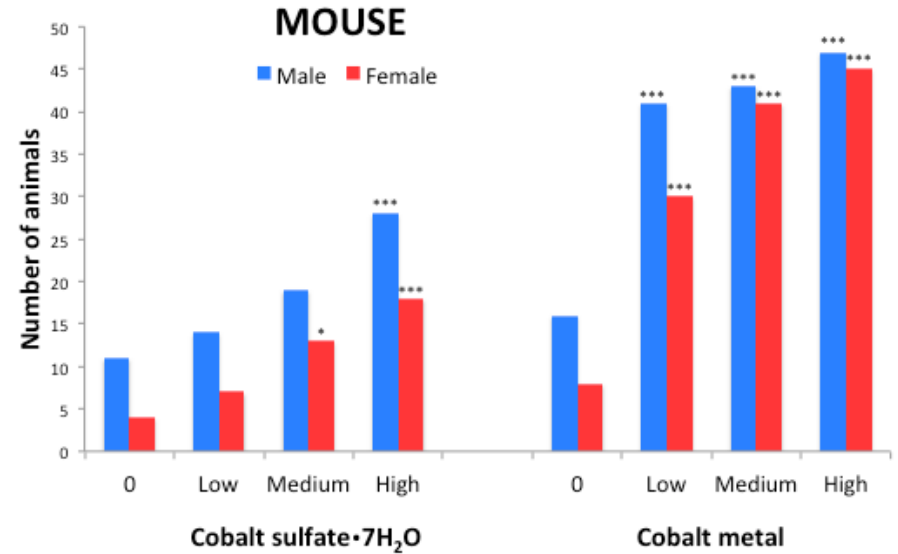
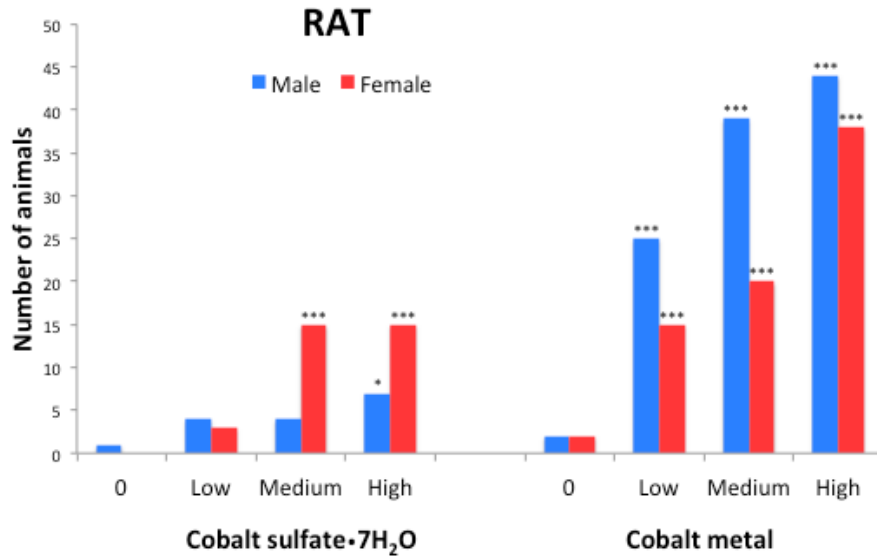


Source: Behl *et al.* 2014



# Inhalation Exposure

## Cobalt induces alveolar/bronchiolar adenoma or carcinoma



- CKE ♀

- Hyperplasia ♂ ♀
- Inflammation ♂ ♀

- Hyperplasia ♂ ♀
- Inflammation ♂ ♀

- Hyperplasia ♂ ♀
- Inflammation ♂ ♀

Individual comparisons: \* $P < 0.05$  \*\*\* $P < 0.001$ .

Trend-test  $P$ -value  $< 0.001$  for all except cobalt sulfate in male rats ( $P = 0.032$ ).

CKE = Cystic keratinizing epithelioma.

Sources: NTP 1998, 2014.



## Cobalt(II) oxide

- Steinhoff & Mohr 1991
  - Intratracheal instillation treatment
    - Male and female rats
    - 0, 2, or 10 mg/kg bw
  - Significant increases in lung neoplasms (alveolar/bronchiolar adenoma or carcinoma) in male rats; non-significant increases were observed in females
- Wehner *et al.* 1977
  - Inhalation treatment
    - Male hamsters
    - 10 mg/m<sup>3</sup>
  - No lung tumors
  - Pneumoconiosis, as evidenced by a variety of lesions
  - Poor survival (thus limited sensitivity)
  - Hamsters less sensitive model (than rats or mice) for detecting lung tumors





## Inhalation exposure to cobalt metal caused significant increases in tumor incidence in rat tissues other than lung

<b>Tissue</b> (Neoplasm)	<b>Findings</b> (Increased incidence)
<b>Pancreas</b> (Islet-cell adenoma or carcinoma, combined)	Male rat: significant increase for top two doses; $P_{trend} = 0.002$ Female rat: findings equivocal (non-significant increase)
<b>Hematopoietic system</b> (Mononuclear-cell leukemia, MCL)	Female rat: significant increases in all exposed groups; increased incidence exceeded historical control for all routes
<b>Kidney</b> (Renal tubule adenoma or carcinoma, combined)	Male rat: findings equivocal (non-significant increase); rare tumor; $P_{trend} = 0.023$ (standard and extended evaluation)

Source: NTP 2014



## Cobalt also induced adrenal gland neoplasms: Benign and malignant pheochromocytoma

- Cobalt metal (NTP 2014)
  - Male and female rats
    - Significant increases in tumor incidence for top two doses,  $P < 0.001$
    - $P_{trend} < 0.001$
- Cobalt sulfate heptahydrate (NTP 1998)
  - Female rats
    - Significant increase in tumor incidence for top dose,  $P < 0.001$
    - $P_{trend} < 0.001$
  - Male rats
    - For mid-dose exposure, incidence significantly greater ( $P < 0.05$ ) than chamber control and exceeded historical control range

It is unclear whether this is a direct systemic effect of cobalt exposure or an indirect secondary response to lung damage (e.g., chronic inflammation).



# Injection Site Tumors in Rats

## Significant increase in tumor incidence at injection site

	Form of Cobalt		
	Water soluble CoCl <sub>2</sub>	Metal Co metal/nano	Poorly water soluble CoO
<b>Injection site tumors</b>			
Sarcoma (all types)	SC	IM, IT	IM, SC, IP
Histiocytoma			SC, IP

- Relevance to humans (e.g., hip replacement)
- Injection study results provide supporting evidence of carcinogenicity of cobalt
  - Consistency of tumor types
  - Similar findings for different forms of cobalt
  - Evidence that tumors are due to carcinogenic properties of cobalt and not just a reaction to physical implant (Hansen *et al.* 2006)
    - Cobalt metal (nanoparticle) and nickel implants induced tumors in rats
    - Titanium dioxide, silicon dioxide, and PVC implants did not induce tumors, although they have the same physical characteristics (i.e., surface to volume ratio)



## Exposure to cobalt causes carcinogenic effects in experimental animals

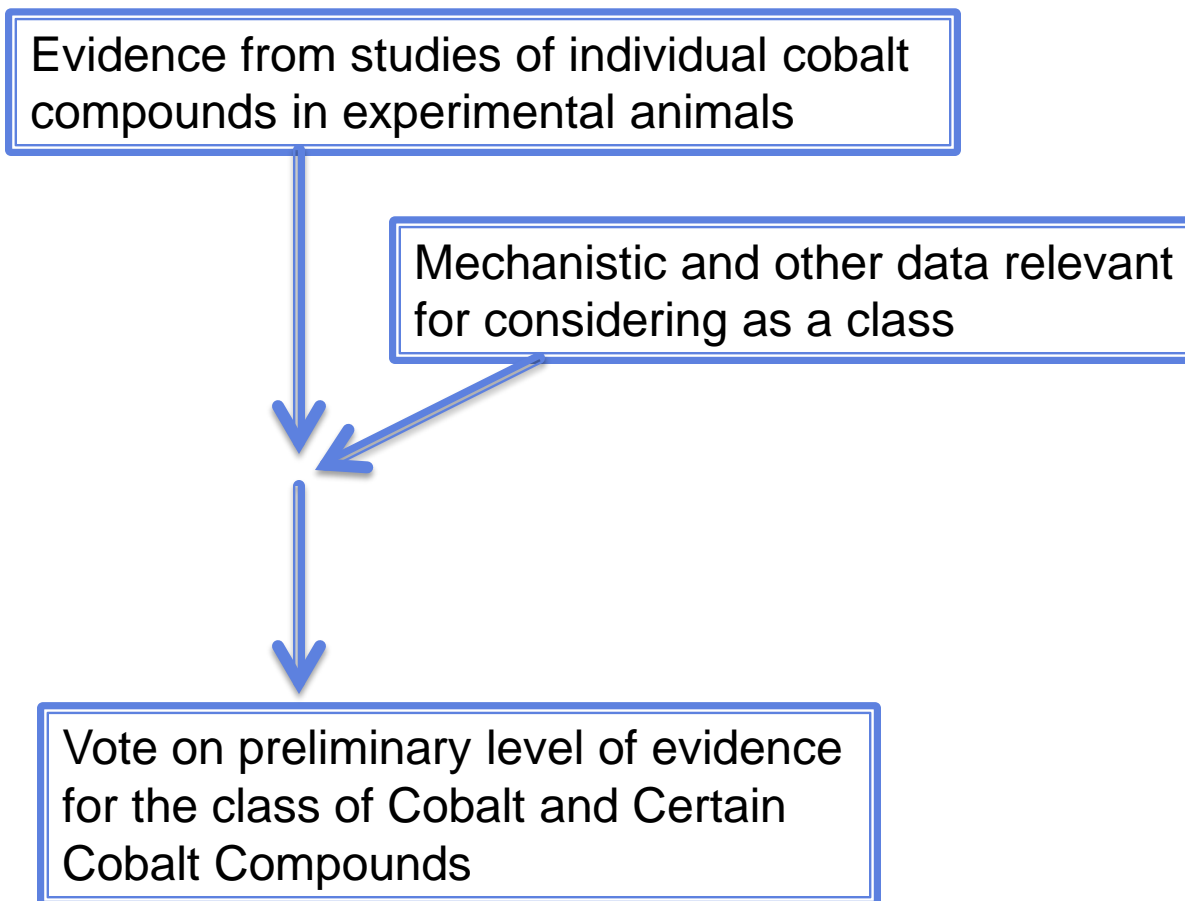
Site	Water soluble ( $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ ; $\text{CoCl}_2$ )	Cobalt metal	Poorly water soluble (CoO)
Lung	Rat ♂♀ Mouse ♂♀	Rat ♂♀ Mouse ♂♀	Rat ♂
Pancreas		Rat ♂(♀)	
MCL		Rat ♀	
Kidney		Rat (♂)	
Adrenal gland	Rat ♀	Rat ♂♀	
Injection site	Rat ♂	Rat ♂♀	Rat ♂♀

( ) = results equivocal

MCL = mononuclear-cell leukemia



## Defer vote on carcinogenicity of cobalt in experimental animals





# Studies in Experimental Animals

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Clarifications?



## Reviewer questions

- Comment on whether the scientific information from cancer studies in experimental animals for cobalt and certain cobalt compounds is clear, technically correct, and objectively presented.
  - Identify any information that should be added or deleted.
- Comment on whether the approach and assessment of the utility of the animal carcinogenicity studies (study quality and sensitivity) for informing the cancer evaluation is systematic, transparent, objective, and clearly presented (Appendix D, Sections 5.1.2 and 5.2.2).
- Provide any scientific criticisms of NTP's cancer assessment of the experimental animal studies of exposure to cobalt and certain cobalt compounds and how findings from the scientific evidence across studies were synthesized.







# Carcinogenicity study quality assessment

## Evaluation on study quality and sensitivity

### Quality

- Population of study animals
  - Controls (concurrent, historical)
  - Randomization of dosing groups
- Quality of exposure
  - Chemical purity
  - Dosing regimen
  - Survival, body weight changes
- Quality of endpoint assessment
  - Pathology (necropsy, histology, diagnosis)
  - Potential for confounding
- Analysis and reporting
  - Statistical analysis
  - Combination of tumor types

### Sensitivity

- Animal model
  - Source
  - Species
  - Strain
  - Sex
- Statistical power
  - number of animals in control or test groups
  - Survival effects (high mortality)
- Study duration
  - Adequate to detect effect, at least one year but better near lifespan of 2 years



## Evaluation of studies for overall utility

- Assessment
  - Potential for bias (limitations)
  - Sensitivity
- Ratings
  - **High**
    - Low/minimal concerns for most potential bias
  - **Moderate**
    - Low/minimal concern or some concerns for most potential bias
  - **Low**
    - Major concerns for several potential biases
  - **Inadequate**
    - Critical concern for some potential bias