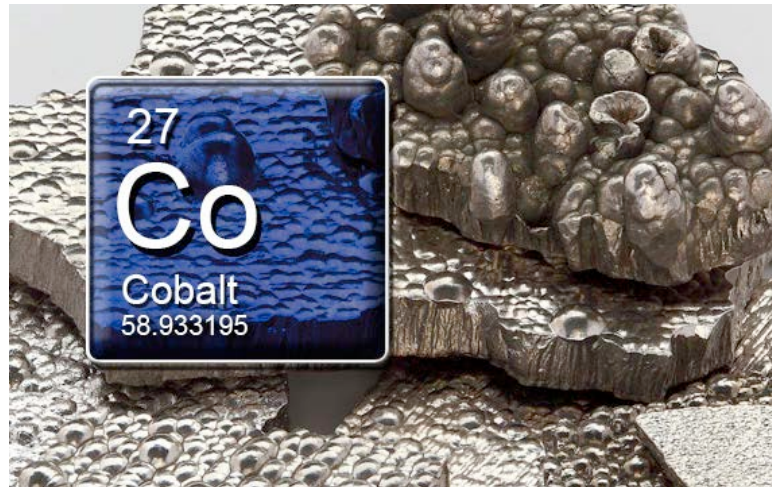# Studies in Experimental Animals

Diane Spencer, MS

Office of the Report on Carcinogens

National Institute of Environmental Health Sciences

July 22, 2015

Outline

Overview of studies in experimental animals

Study quality assessment

Cancer assessment

– Inhalation tumors in lung and other sites
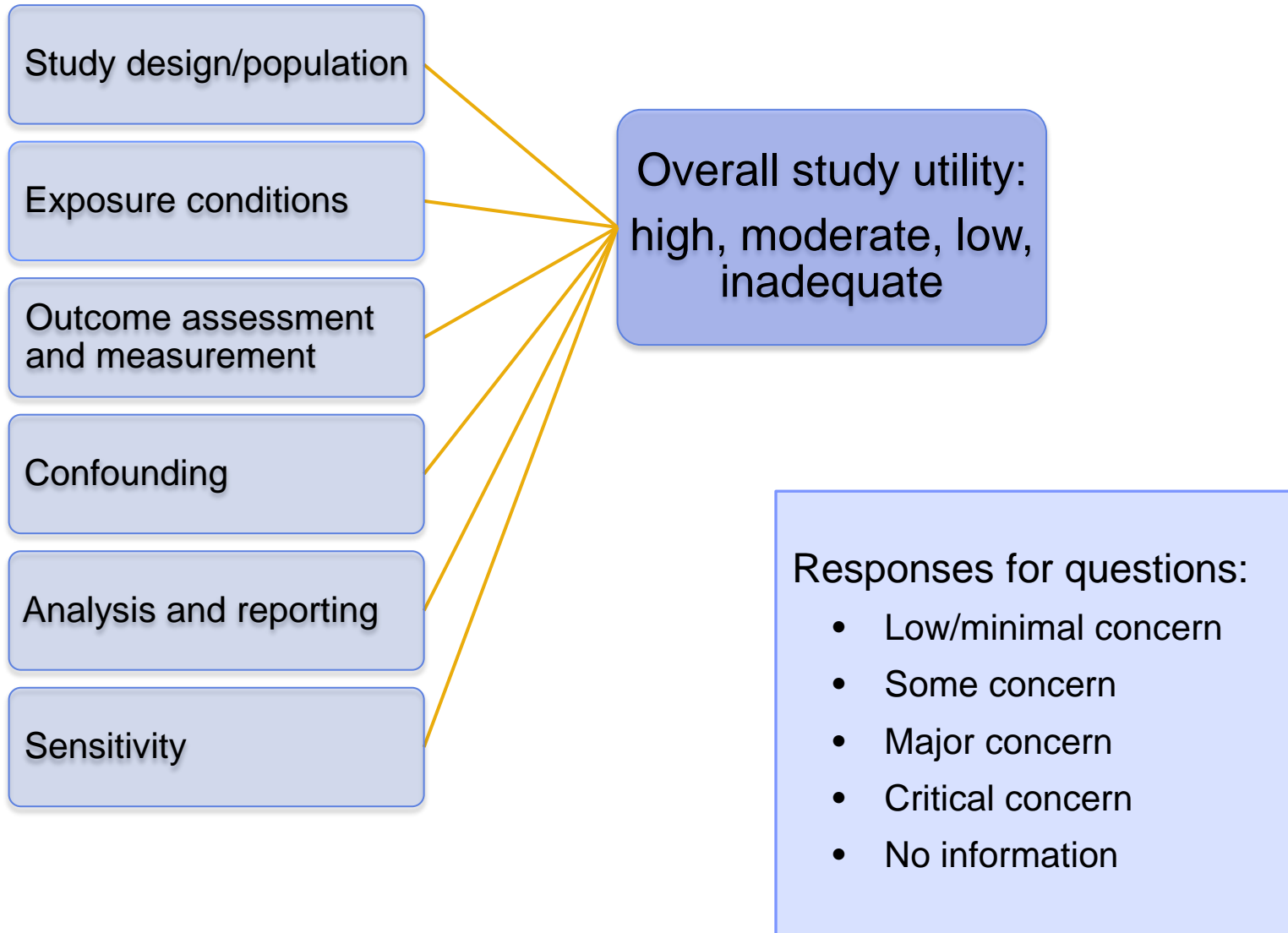– Injection site tumors

Carcinogenicity Studies

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

Study design/population

Exposure conditions

Outcome assessment and measurement

Confounding

Analysis and reporting

Sensitivity

Overall study utility:
high, moderate, low, inadequate

Responses for questions:
- Low/minimal concern
- Some concern
- Major concern
- Critical concern
- No information

Carcinogenicity Study Quality Assessment

Overall study utility

Overall Utility	Studies
High	NTP 1998 (rat and mouse) NTP 2014 (rat and mouse)
Moderate	Hansen *et al.* 2006 Steinhoff & Mohr 1991 (3 routes) Wehner *et al.* 1977
Low	Gilman & Ruckerbauer 1962 (rat and mouse) Heath 1956 Heath & Daniel 1962 Jasmin & Riopelle 1976 Shabaan *et al.* 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
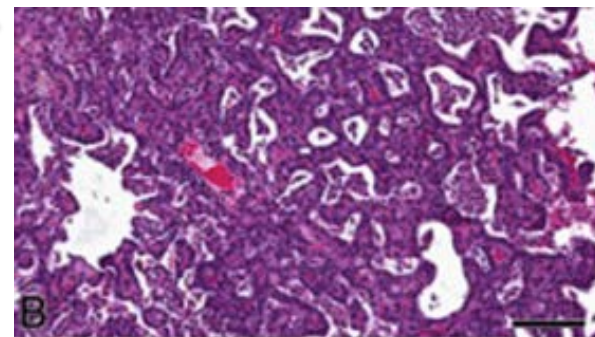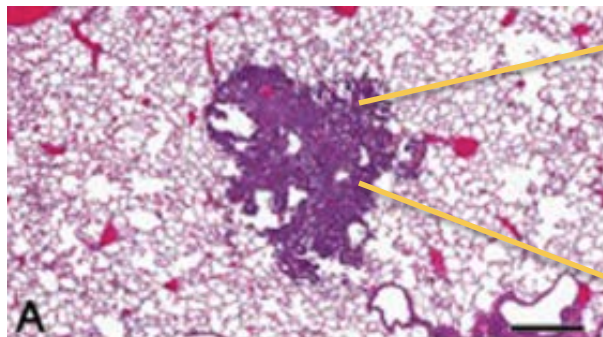
Different types of cobalt compounds caused lung neoplasms after exposure in rodents

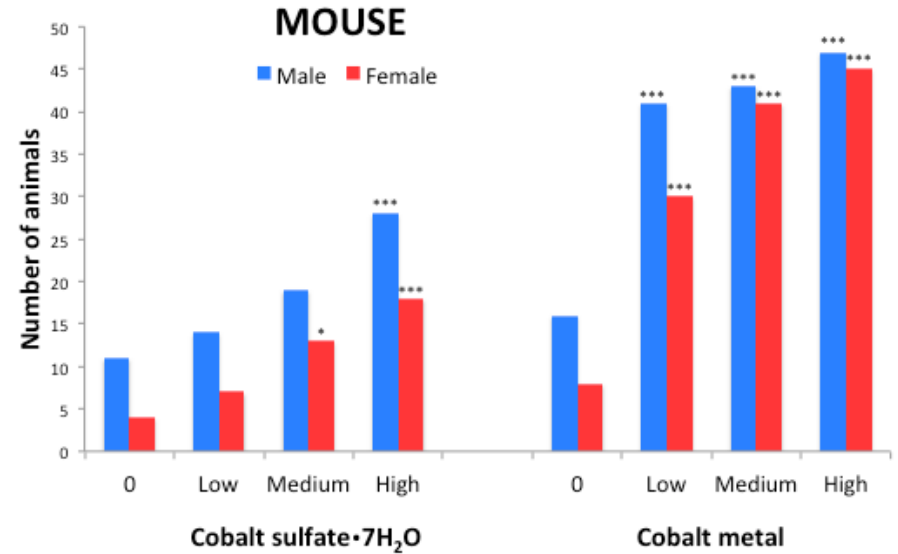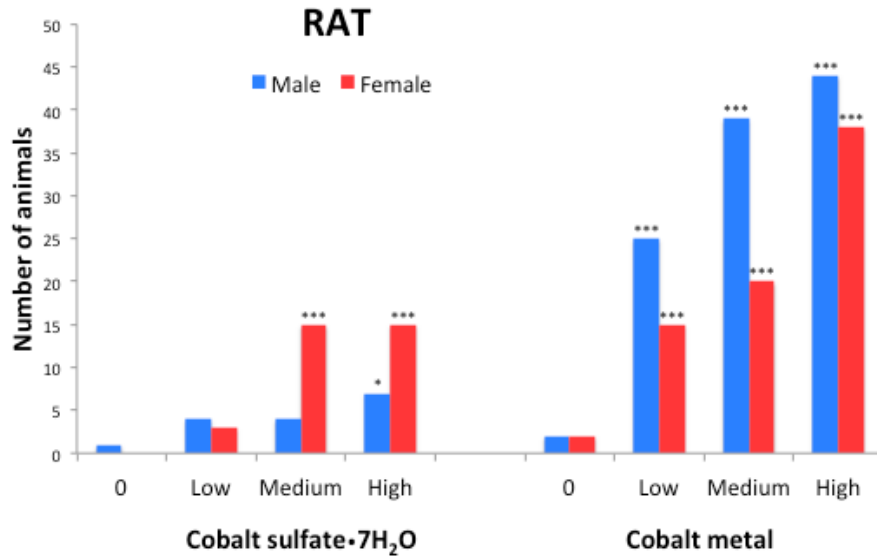|	RAT		MOUSE	
Cobalt form	Male	Female	Male	Female
Water soluble ($CoSO_4 \cdot 7H_2O$)	✔	✔	✔	✔
Metal	✔	✔	✔	✔
Poorly water soluble (CoO)	✔	✔	NT	NT

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

Source: Behl *et al.* 2014

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^3
  - 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

Tissue (Neoplasm)	Findings (Increased incidence)
Pancreas (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)
Hematopoietic system (Mononuclear-cell leukemia, MCL)	Female rat: significant increases in all exposed groups; increased incidence exceeded historical control for all routes
Kidney (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).

Significant increase in tumor incidence at injection site

Injection site tumors	Form of Cobalt		
	Water soluble	**Metal**	**Poorly water soluble**
	$CoCl_2$	Co metal/nano	CoO
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)

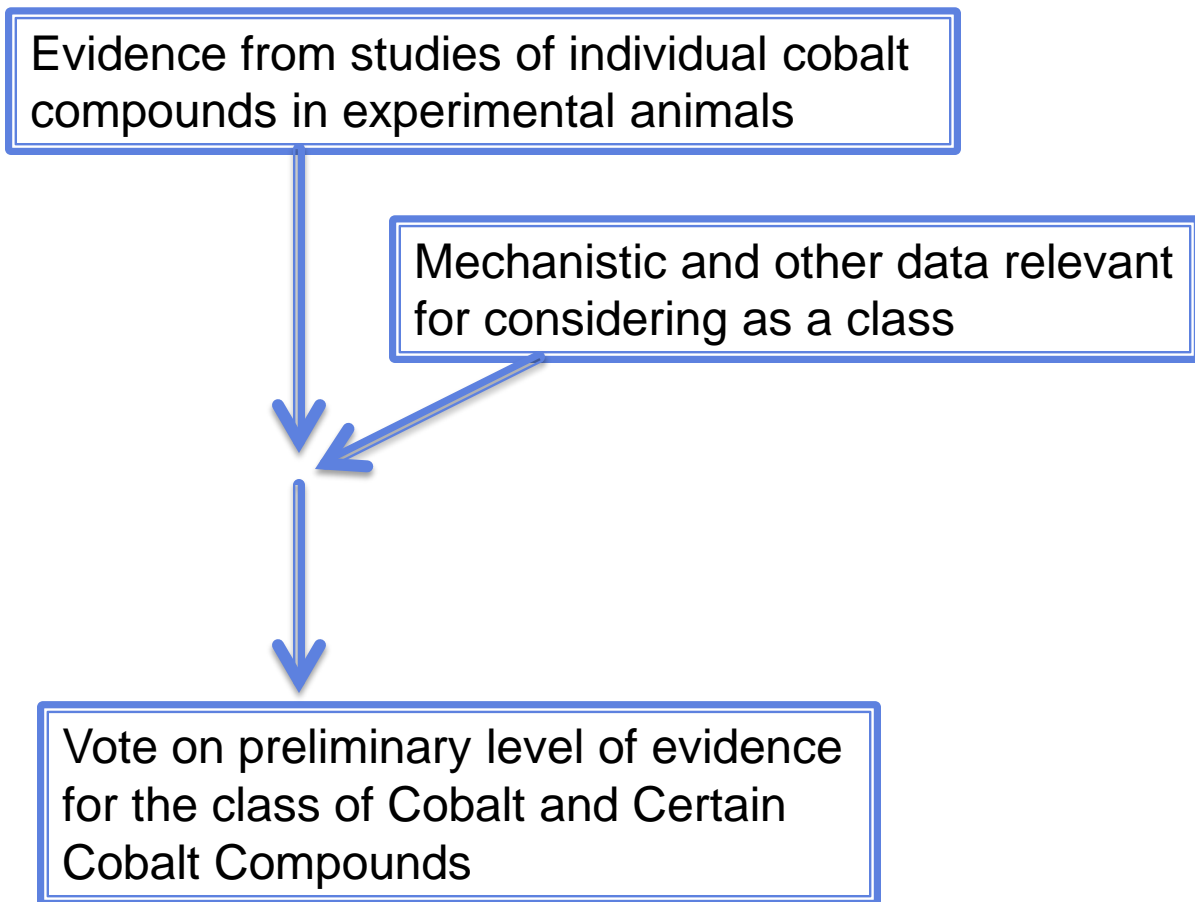## Exposure to cobalt causes carcinogenic effects in experimental animals

Site	Water soluble (CoSO$_4$•7H$_2$O; 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

Evidence from studies of individual cobalt compounds in experimental animals

Mechanistic and other data relevant for considering as a class

Vote on preliminary level of evidence for the class of Cobalt and Certain Cobalt Compounds

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