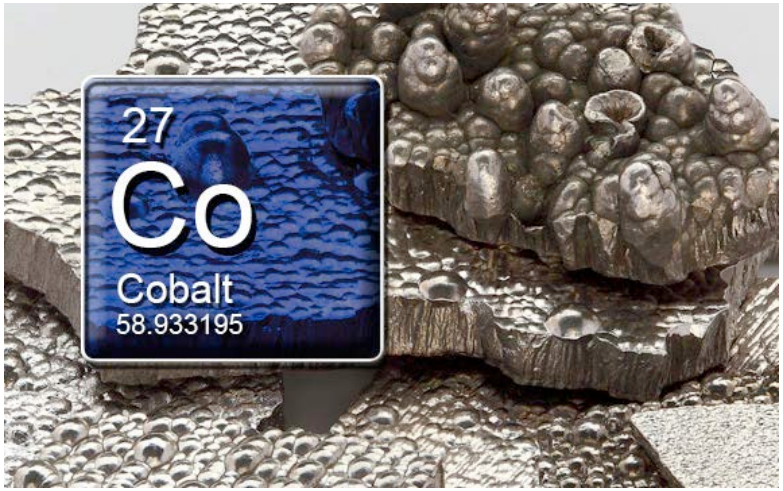
Mechanistic and Other Relevant Data



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National Institute of Environmental Health Sciences

July 22, 2015

Mechanistic and Other Relevant Data

Outline

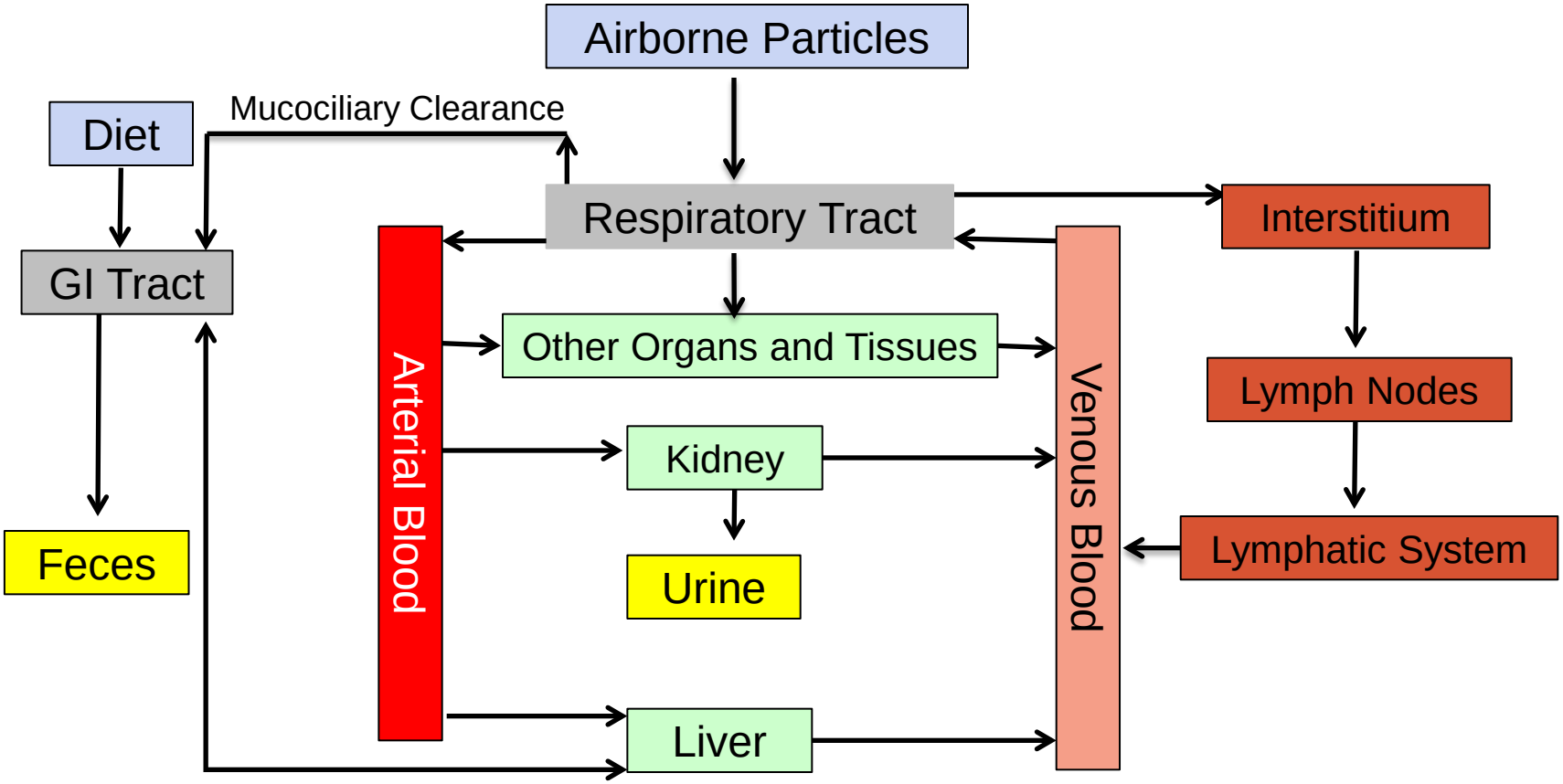
Disposition and Toxicokinetics

Mechanistic and Other Relevant Data

- Cobalt toxicity: particles versus ions
- Proposed modes of action
- Cobalt and certain cobalt compounds as a class

Cobalt Disposition and Toxicokinetics

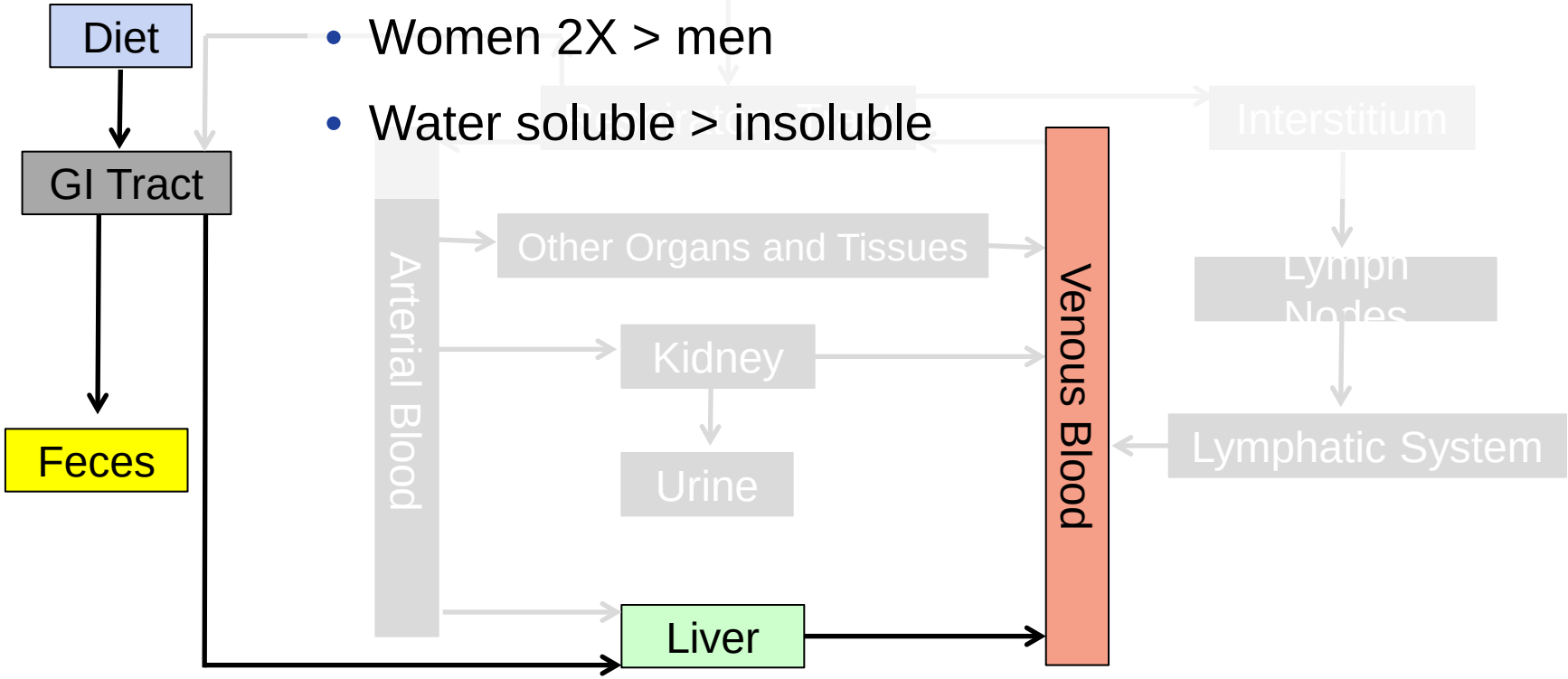
Diet and inhalation are important exposure pathways



Dietary Exposure

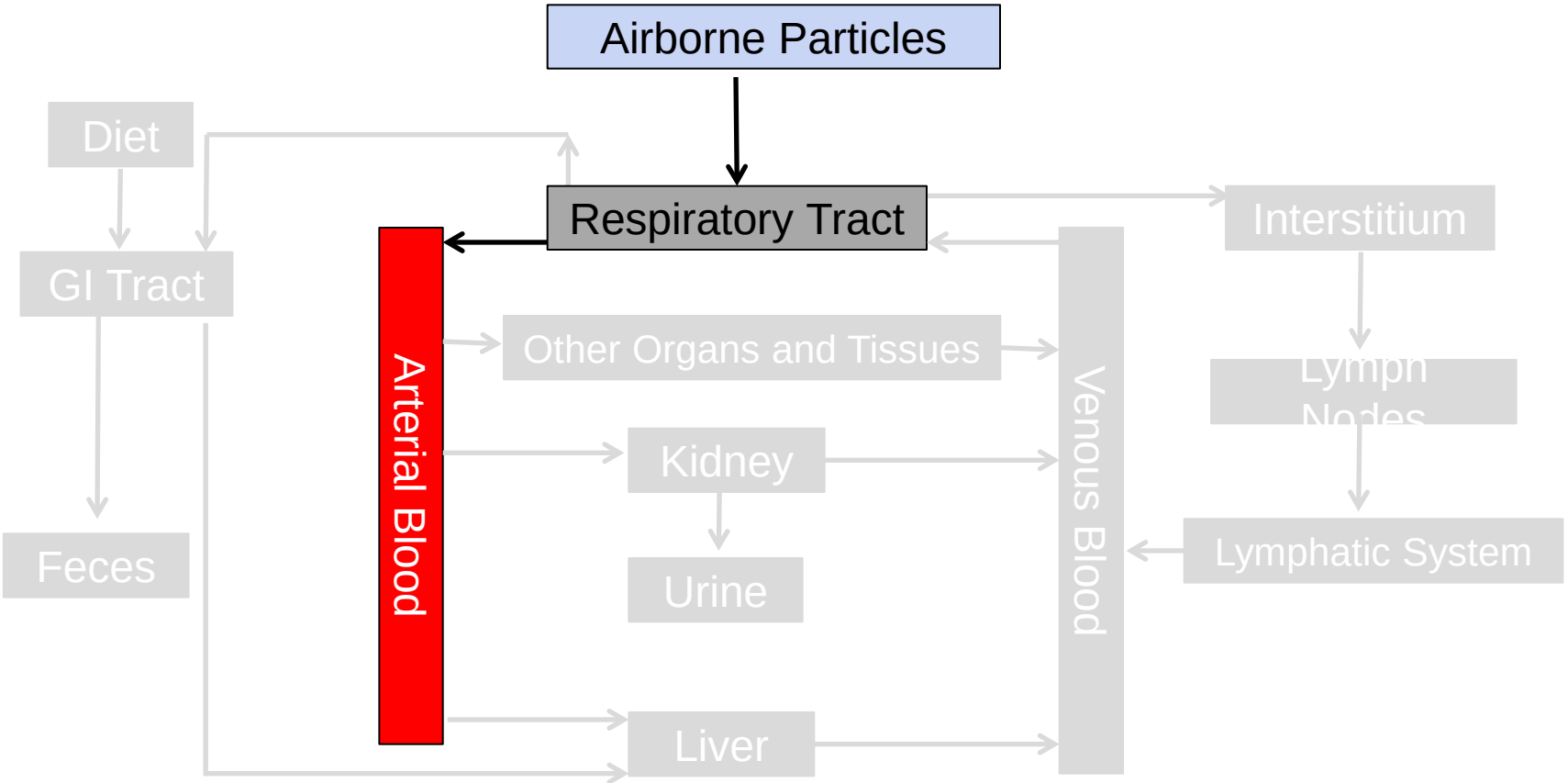
Absorption from the GI tract is highly variable (5% to > 90%)

- Aqueous (20% to 45%)
- Solid (10% to 25%)
- Women 2X > men
- Water soluble > insoluble



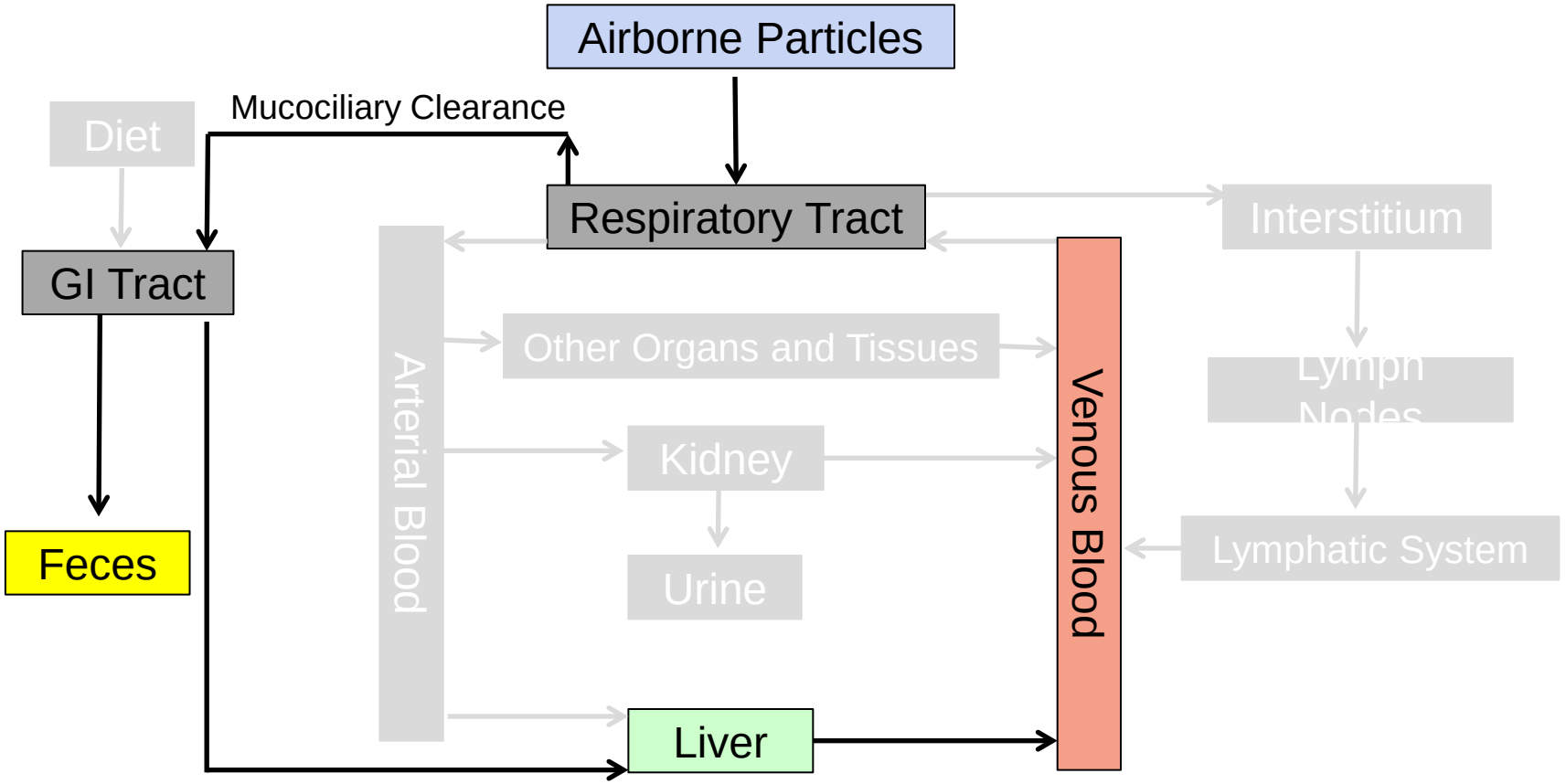
Inhalation Exposure

Soluble compounds/particles are rapidly absorbed



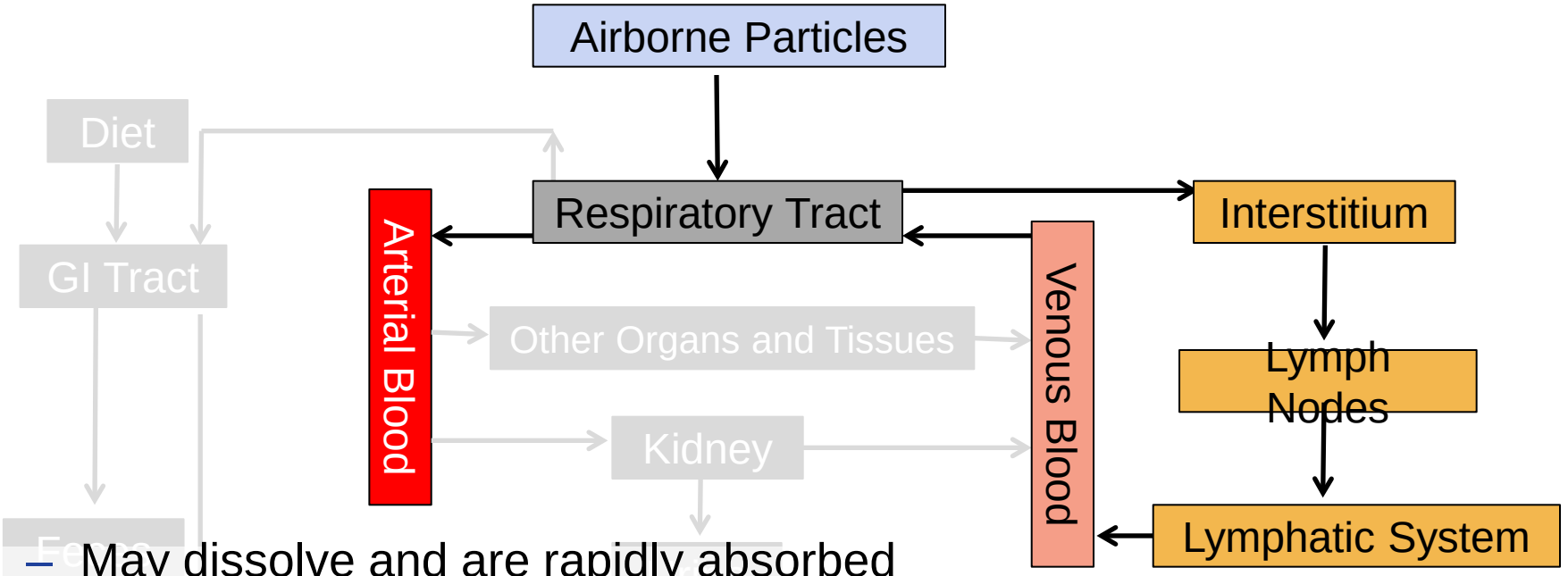
Inhalation Exposure

Large particles cleared by mucociliary action



Inhalation Exposure

Smaller particles reach the lower airways

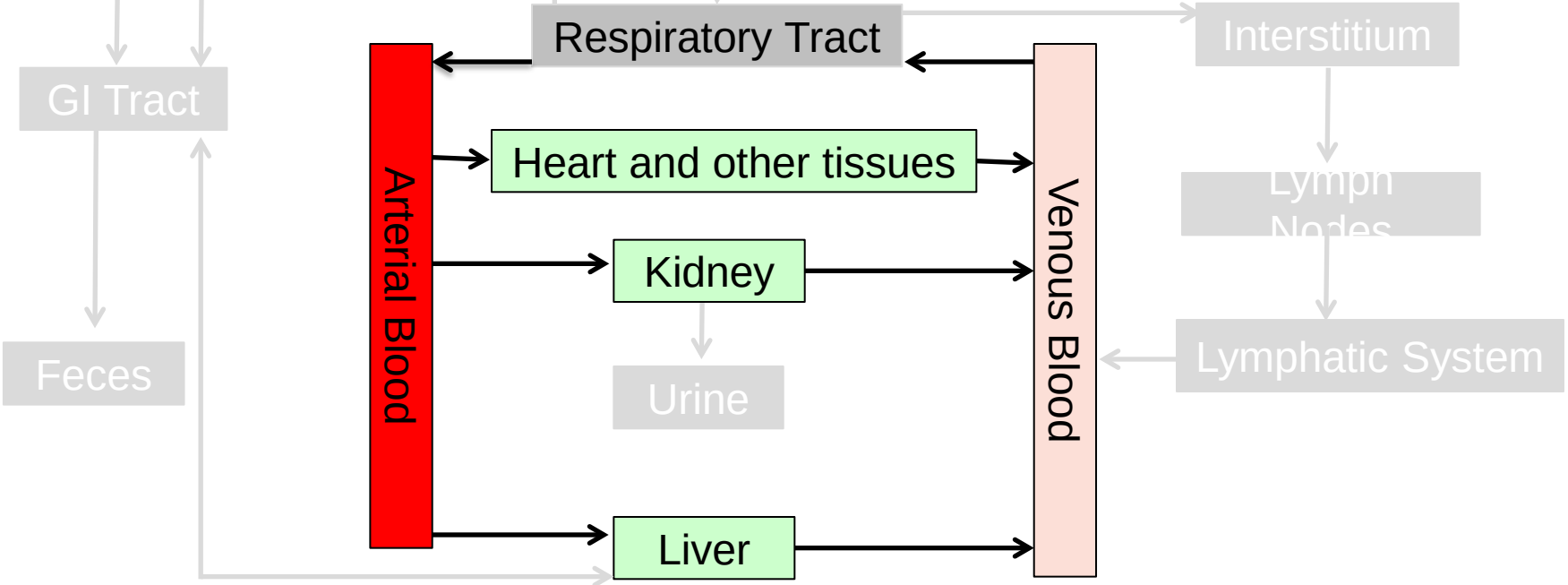


- May dissolve and are rapidly absorbed
- Phagocytized by alveolar macrophages
- Enter lung cells via endocytosis
- Nanoparticles may translocate directly to blood and lymph

Cobalt Disposition

Absorbed cobalt rapidly distributes to all tissues

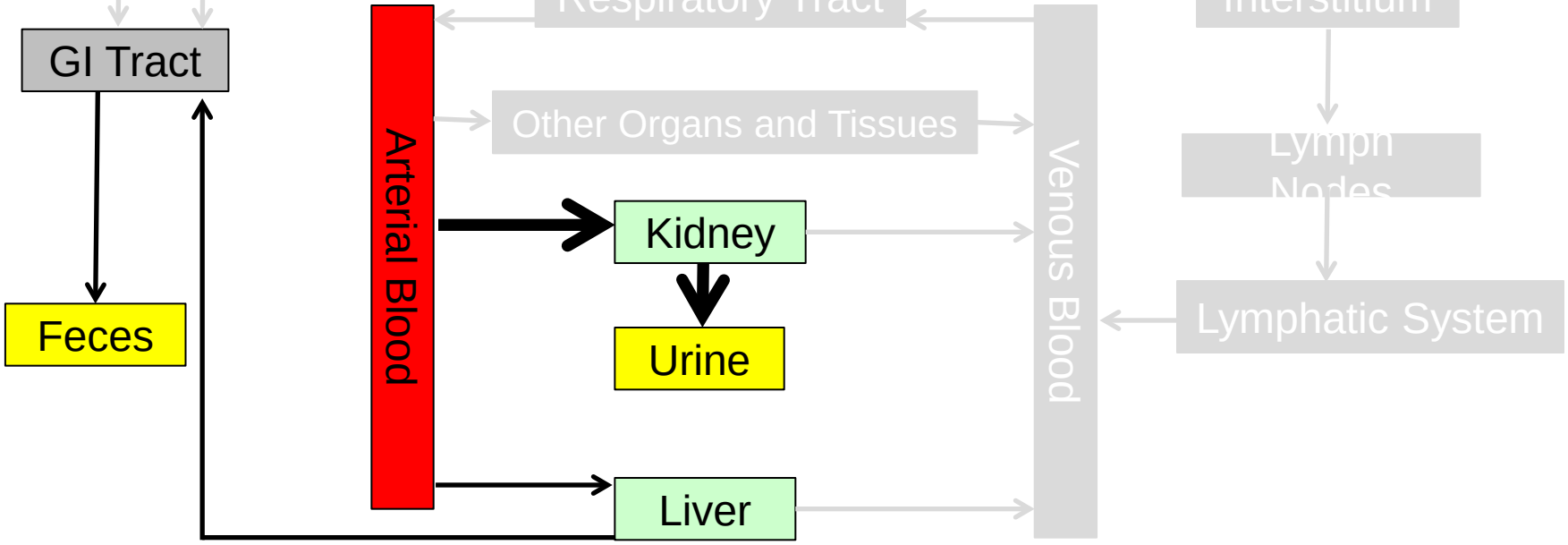
- Highest in liver, kidney, heart, spleen
  - Stored body Co does not significantly accumulate with age
- Free fraction in blood (5% to 12%)
- Insoluble particles/compounds may be retained in the lungs



Cobalt Excretion

Much of absorbed cobalt is excreted within the first week

- Bile → feces (2% to 12%)
- Urine (30% to 60%)
- Multiphasic elimination



Clarifications?

Reviewer Comments

- Comment on whether the information on disposition and toxicokinetics is clear, technically correct, and objectively presented.
- Identify any information that should be added or deleted.

Outline

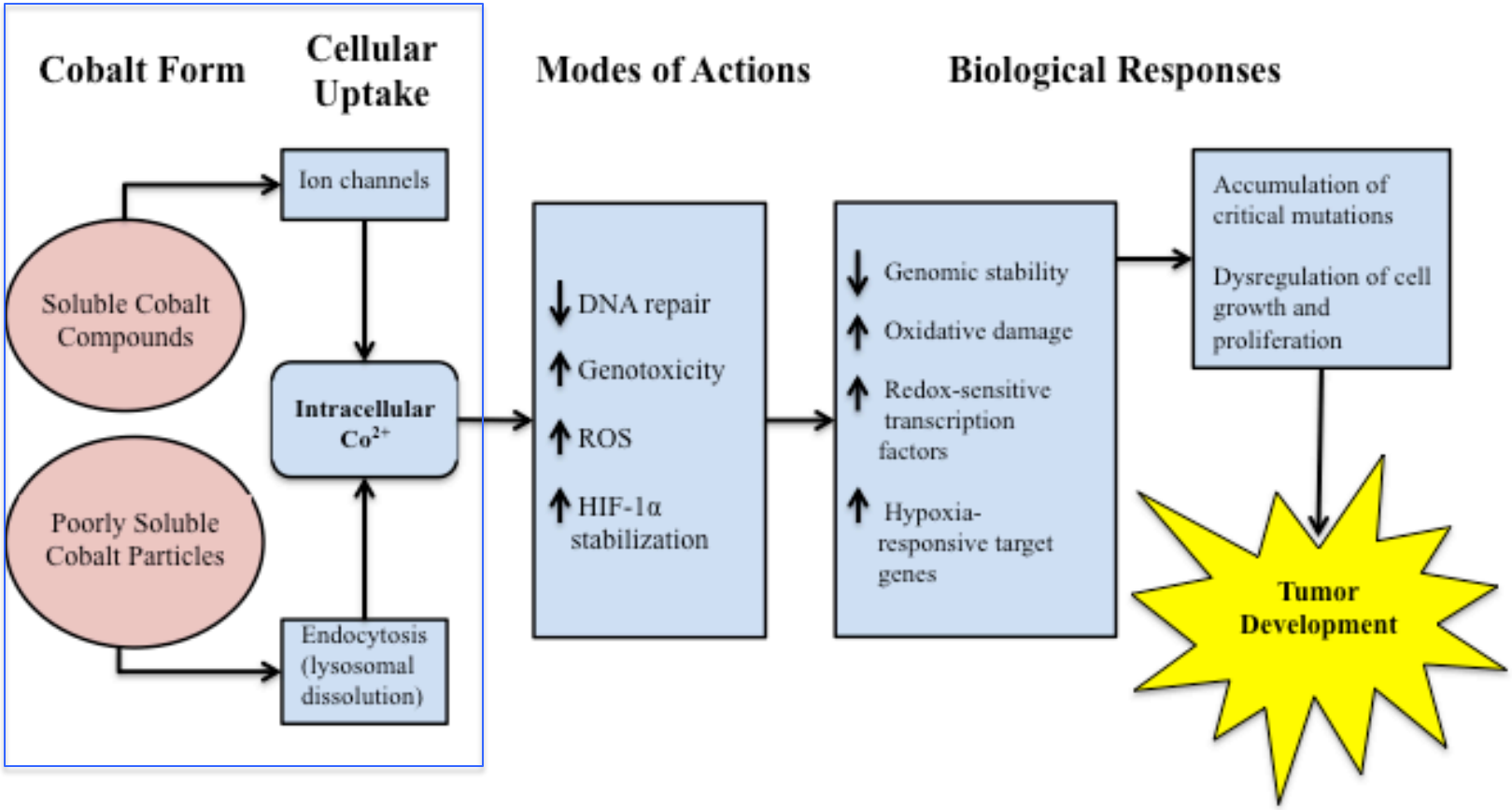
Disposition and Toxicokinetics

Mechanistic and Other Relevant Data

- Cobalt toxicity: particles versus ions
- Proposed modes of action
- Cobalt and certain cobalt compounds as a class

Potential Modes of Action

Cobalt and cobalt compounds affect similar pathways associated with carcinogenesis



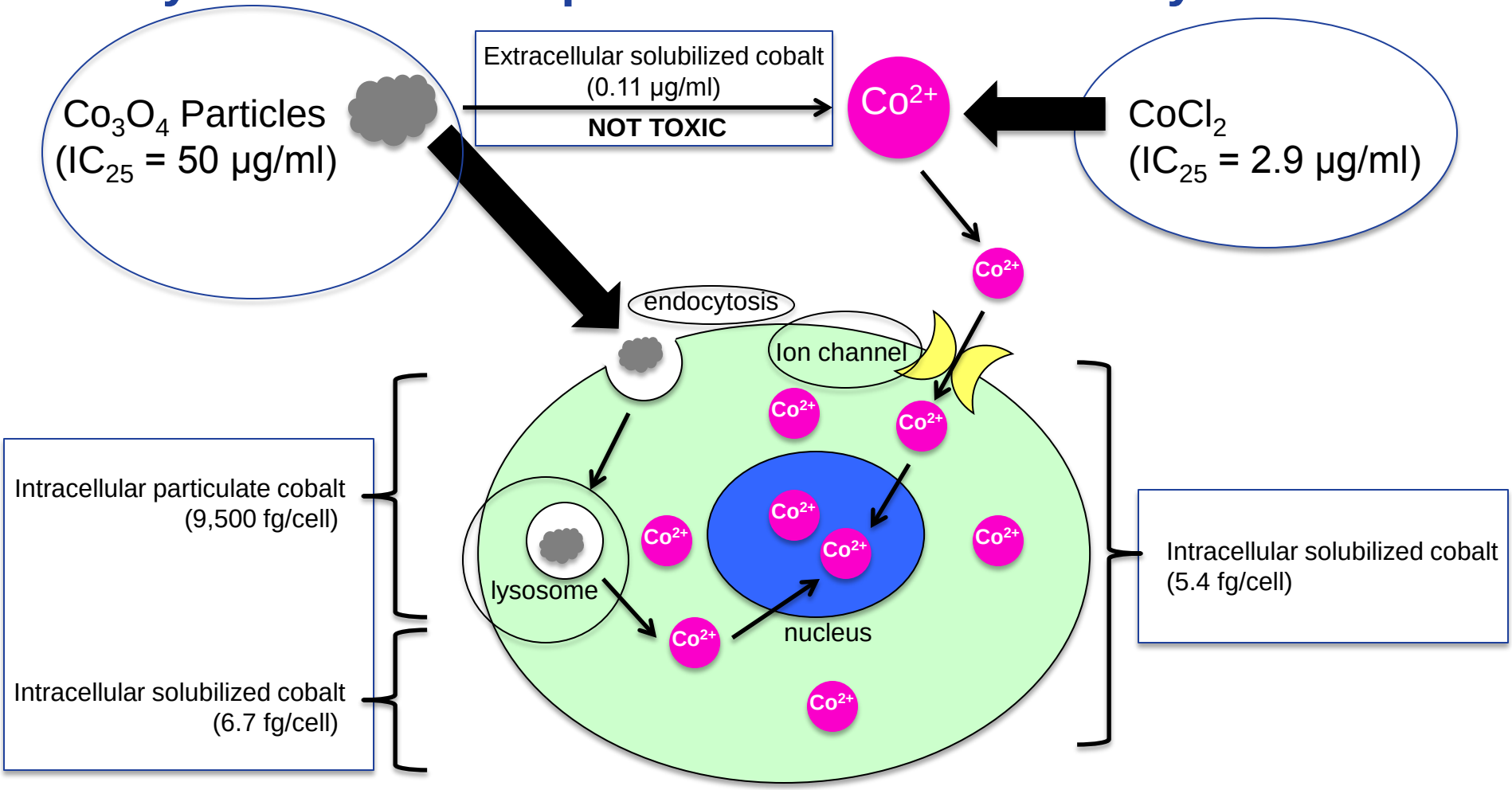
Mechanistic and Other Relevant Data

Cobalt particles and ions have similar biological effects *in vitro* and *in vivo*

- Cytotoxicity/Inflammation
  - Nanoparticles > microparticles
  - Relatively soluble particles > ions > poorly soluble particles
- ROS
  - Nano- and microparticles > ions
  - Nanoparticles activate neutrophils: ROS/proinflammatory cytokines
- For particles, direct cell contact was necessary to induce effects
- Co^{2+} ions responsible for effects, differences partially explained by cellular uptake mechanisms

Cellular Uptake of Cobalt Ions and Particles

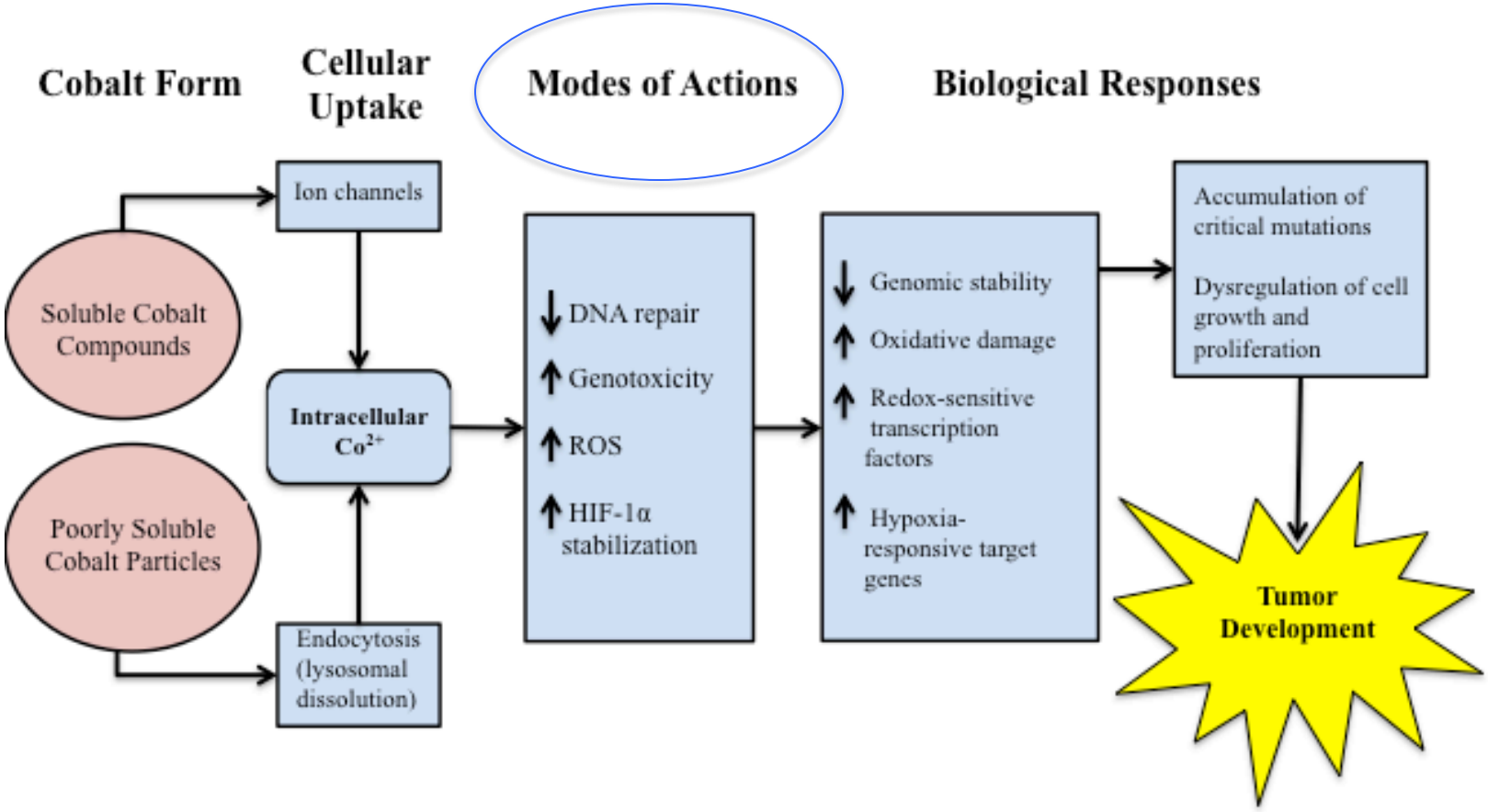
Poorly soluble cobalt particles dissolve in the lysosomes



Source: Ortega et al. 2014

Potential Modes of Action

Cobalt and cobalt compounds affect similar pathways associated with carcinogenesis



Cobalt and cobalt compounds induced a similar spectrum of genotoxic and related effects

- *In vitro* studies generally consistent for all forms tested
  - Bacteria: mostly negative
  - Human and rodent cells
    - Clastogenic (CA, MN, SCE, aneuploidy)
    - DNA damage/strand breaks
    - Mutations (mixed results)
    - Cell transformation
- *In vivo* (few studies)
  - Mixed: Some evidence DNA/chromosome damage in rodents
  - Humans (inadequate data)

Cobalt inhibits DNA repair

- Inhibits nucleotide excision repair (NER)
  - Inhibits incision and polymerization steps of NER
  - Increased DNA damage from UV exposure
- Substitute for Zn(II) in zinc finger domains of DNA-repair proteins and transcription factors
  - p53 (repair of oxidative damage)
  - Xeroderma pigmentosum group A (XPA)
  - Poly(ADP-ribose)polymerase (PARP)
- Competition with Mg(II) for binding to DNA polymerases

Cobalt is a redox-active metal

(1) $Co(0) + O_2 \longrightarrow Co(I) + O_2^{\bullet} \longrightarrow Co(I)-OO^{\bullet}$

(2) $Co(I)-OO^{\bullet} \xrightarrow{SOD} Co(I) + H_2O_2$

(3) $Co(I) + H_2O_2 \longrightarrow Co(II) + {}^{\bullet}OH + OH^-$

(4) $[Co(II)-chelate] + H_2O_2 \longrightarrow Co(II) + {}^{\bullet}OH + OH^-$

Sources: Lee *et al.* 2012, Jomova and Valko 2011, Leonard *et al.* 1998

ROS activate redox transcription factors

- NF-κB
  - Activation is linked to cancer
  - Binds DNA: regulates cell growth & survival, differentiation, cytokine production, inflammation, angiogenesis
- Activator protein-1 (AP-1)
  - Participates in oncogenic transformation
  - Regulates cell proliferation, apoptosis
- Promotes tumor growth by dysregulation of cell growth, proliferation, and survival

Cobalt and cobalt compounds cause oxidative stress/damage

- Cobalt salts (inorganic and organic) strongly active in Nrf2/ARE assay (Tox21)
- Increased sensitivity of 8-oxoguanine-DNA glycosylase (*Ogg*) knockout mouse embryo fibroblasts
- Cobalt particles induced dose-dependent increase in ROS in human and animal cells *in vitro*
- Evidence of oxidative damage *in vivo* in rodents (lung, liver, kidney)
- Lung tumors in rodents induced by cobalt sulfate and cobalt metal had increased frequency of G to T transversion mutations in K-*ras*

Cobalt and cobalt compounds stabilize HIF-1α

- Cobalt well known to mimic hypoxia *in vitro* and *in vivo*
  - Soluble cobalt salts ($CoCl_2$ and $CoSO_4$)
  - Cobalt metal nanoparticles
  - Poorly soluble cobalt oxide (Co_3O_4)
- Possible mechanism(s)
  - Cobalt replaces iron in regulatory oxygenases, prevents hydroxylation of HIF and subsequent ubiquitination and degradation
  - Depletes intracellular ascorbate, deactivates prolyl hydrolase activity, prevents hydroxylation of HIF

Cellular effects of HIF-1α stabilization

- Regulates > 100 hypoxia-responsive genes
  - VEGF, other angiogenic growth factors
  - Erythrocytosis
  - Inflammatory factors
  - Cell proliferation
  - Apoptosis
- Major role in adaption of cancer cells to hypoxia
- Overexpression/stabilization in more that 70% of human cancers and associated with poor clinical outcome.

Rationale for evaluating cobalt and certain cobalt compounds as a class

- Toxicity for all cobalt forms tested attributed primarily to the cobalt ion
- Cobalt metal and water soluble and poorly water soluble cobalt compounds release cobalt ions in biological fluids and have similar biological effects

Cobalt and Cobalt Compounds as a Class

Similar properties and biological effects

Endpoint	Soluble cobalt salts		Cobalt metal	Poorly soluble cobalt compounds
	$CoCl_2$	$CoSO_4$	Particles	CoO or Co_3O_4
Bioacessibility				
Lysosome*	+	+	+	+
Gastric	+	+	+	+
Cellular uptake	+	+	+	+
Cytotoxicity	+	+	+	+
ROS	+	ND	+	+
HIF-1α stabilization	+	+	+	+
DNA repair inhibition	+	ND	+	ND
Genotoxicity *in vitro*	+	+	+	+
Genotoxicity *in vivo*	+	ND	–	ND

ND = no data

* Dissolution of cobalt particles in lysosomal fluid is a key component for the proposed mechanisms

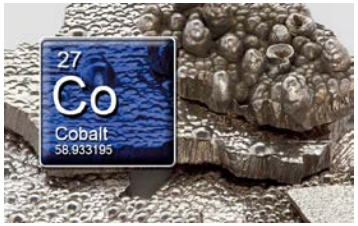
Cobalt and Cobalt Compounds as a Class

In vivo studies of cobalt sulfate and cobalt metal support common underlying mechanisms

Cobalt sulfate →

- Carcinogenic to lung and adrenal gland
- K-*ras* mutation spectra in lung tumors
- Spectrum of inflammatory lesions in lung and extrapulmonary effects in rats
- Similar clinical findings (erythrocytosis)

← Cobalt metal



Source: Behl *et al.* 2015, NTP 2014, 1998

Cobalt and Cobalt Compounds as a Class

Cobalt and cobalt compounds induced similar carcinogenic effects in rodents

Animal Neoplasms	Soluble cobalt salts		Cobalt metal*	Poorly soluble cobalt compounds
	$CoCl_2$	$CoSO_4$	particles	CoO
Lung	ND	+	+	+
Adrenal gland	ND	+	+	ND
Injection site	+	ND	+	+

ND = no data

* Cobalt metal

- Pancreatic islet tumors (exposure related)
- Mononuclear cell leukemia (exposure related)
- Kidney tumors (equivocal)

Clarifications?

Mechanistic and Other Relevant Data

Reviewer Comments

- Comment on whether the mechanistic and other relevant data are clear, technically correct, and objectively presented.
- Comment on whether the mechanistic and other relevant data are appropriate for evaluating the biological plausibility of carcinogenic effects of cobalt and certain cobalt compounds in humans.
- Comment on whether the rationale for evaluating cobalt and certain cobalt compounds as a class is clear and scientifically sound.
- Identify any information that should be added or deleted.