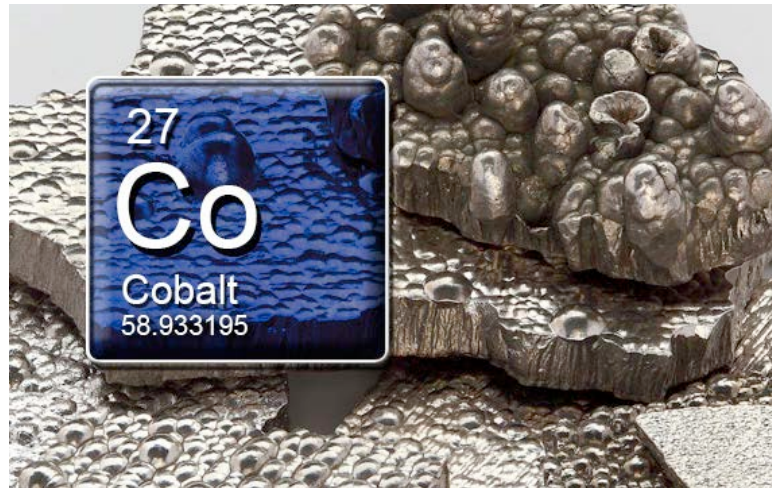


Mechanistic and Other Relevant Data



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Integrated Laboratory Systems, Inc.
Contractor supporting the ORoC

National Institute of Environmental Health Sciences
July 22, 2015



Outline

Disposition and Toxicokinetics

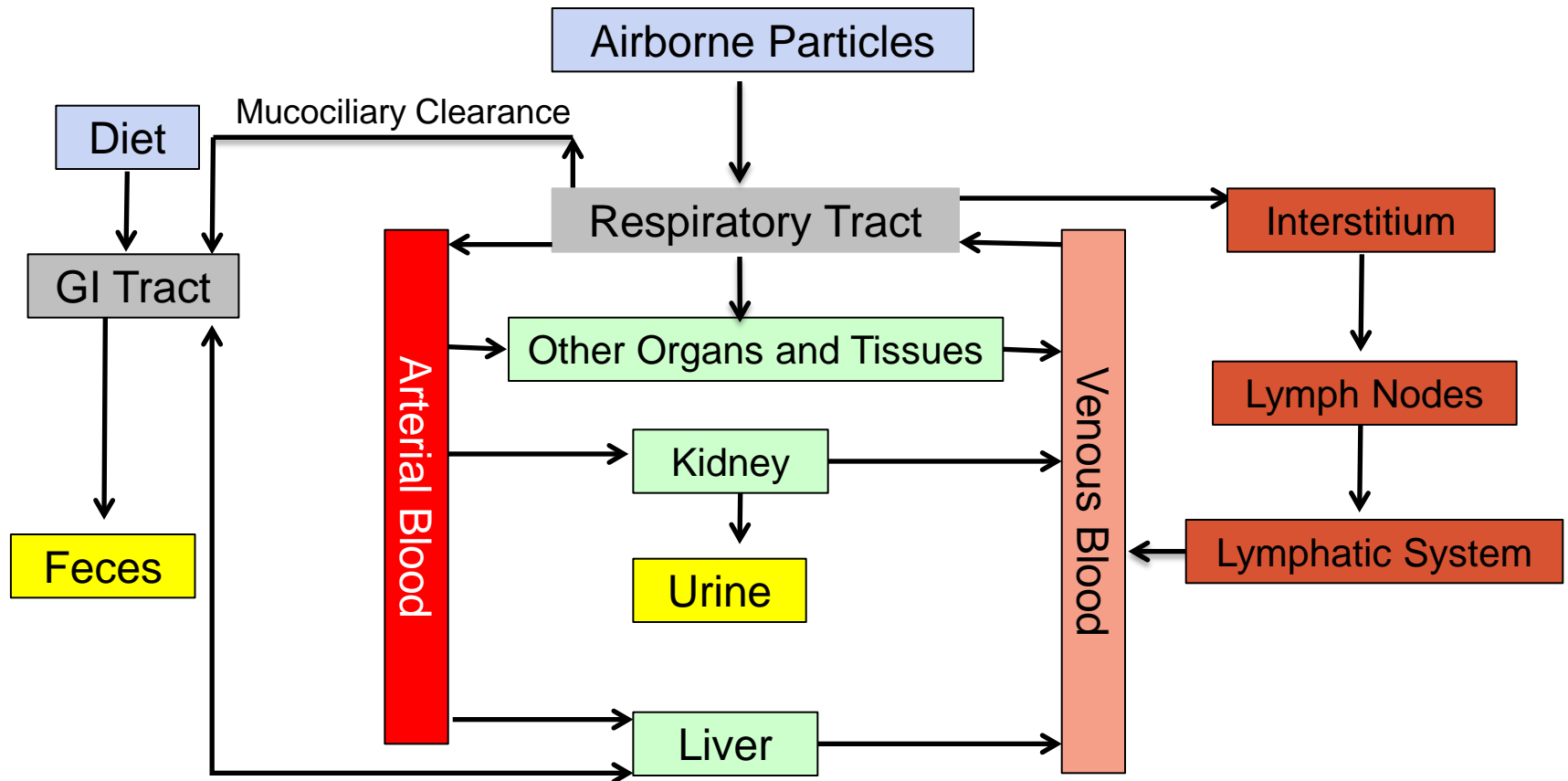
Mechanistic and Other Relevant Data

- 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

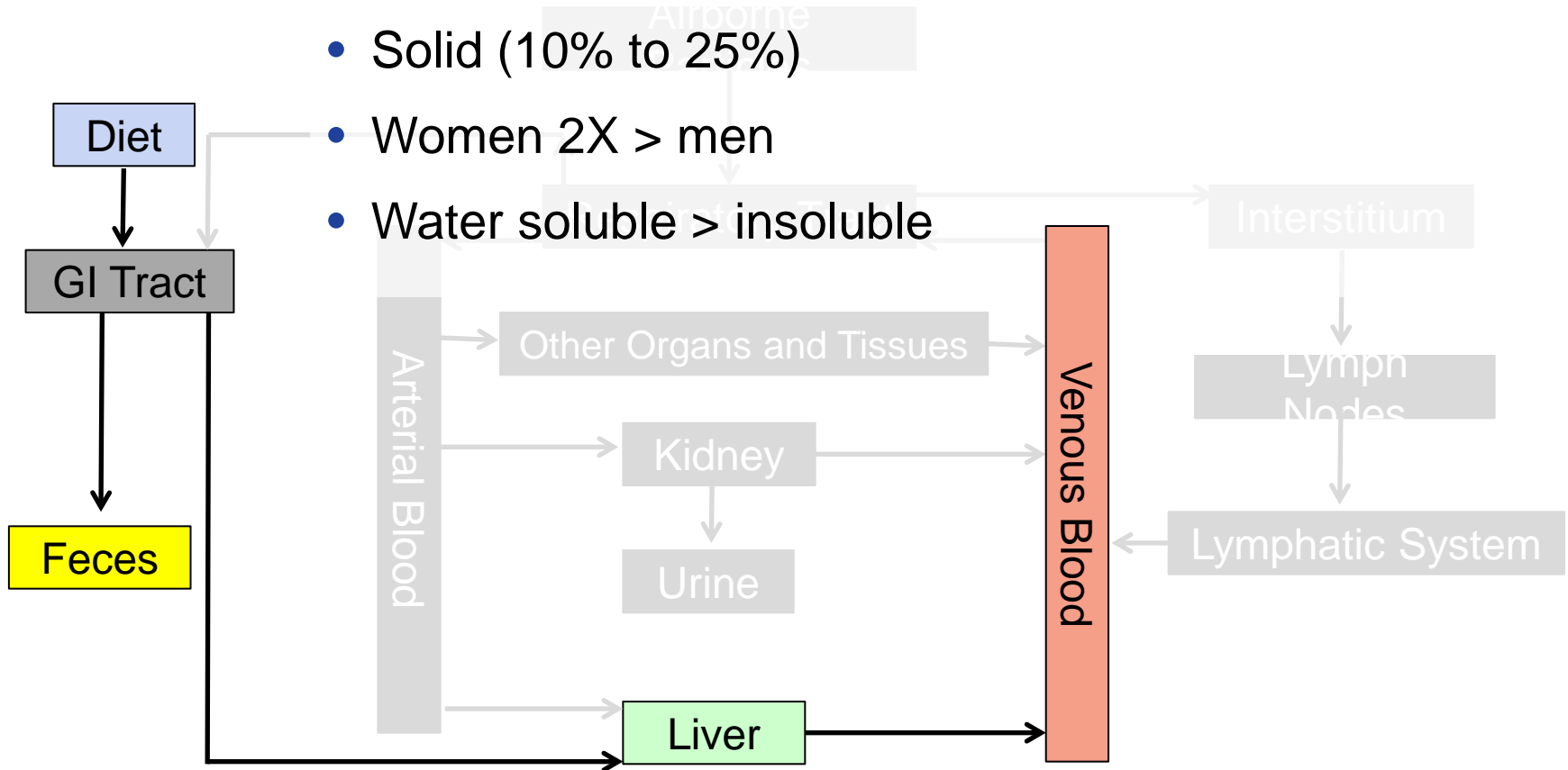




Dietary Exposure

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

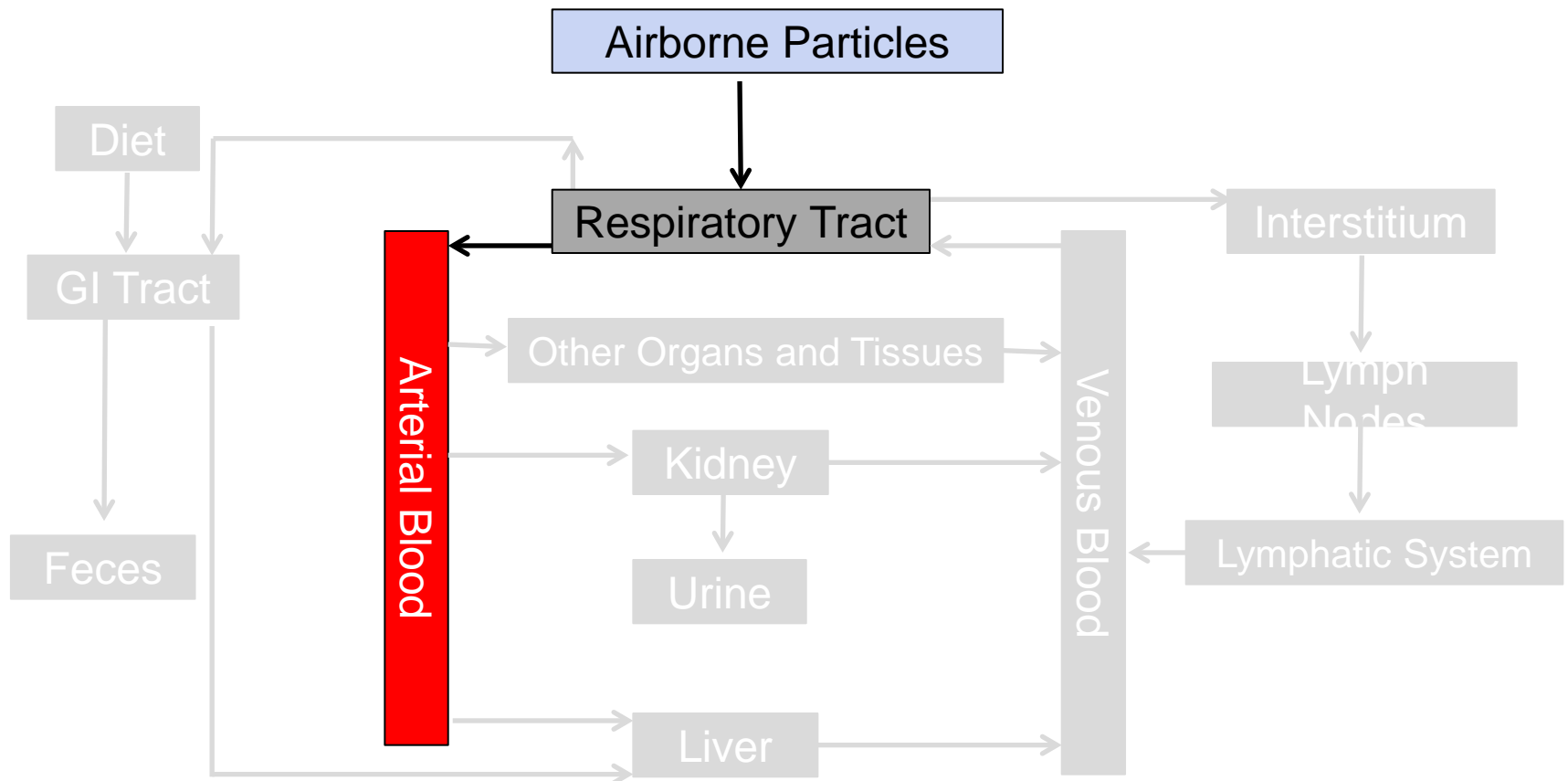
- Aqueous (20% to 45%)
- Solid (10% to 25%)
- Women 2X > men
- Water soluble > insoluble





Inhalation Exposure

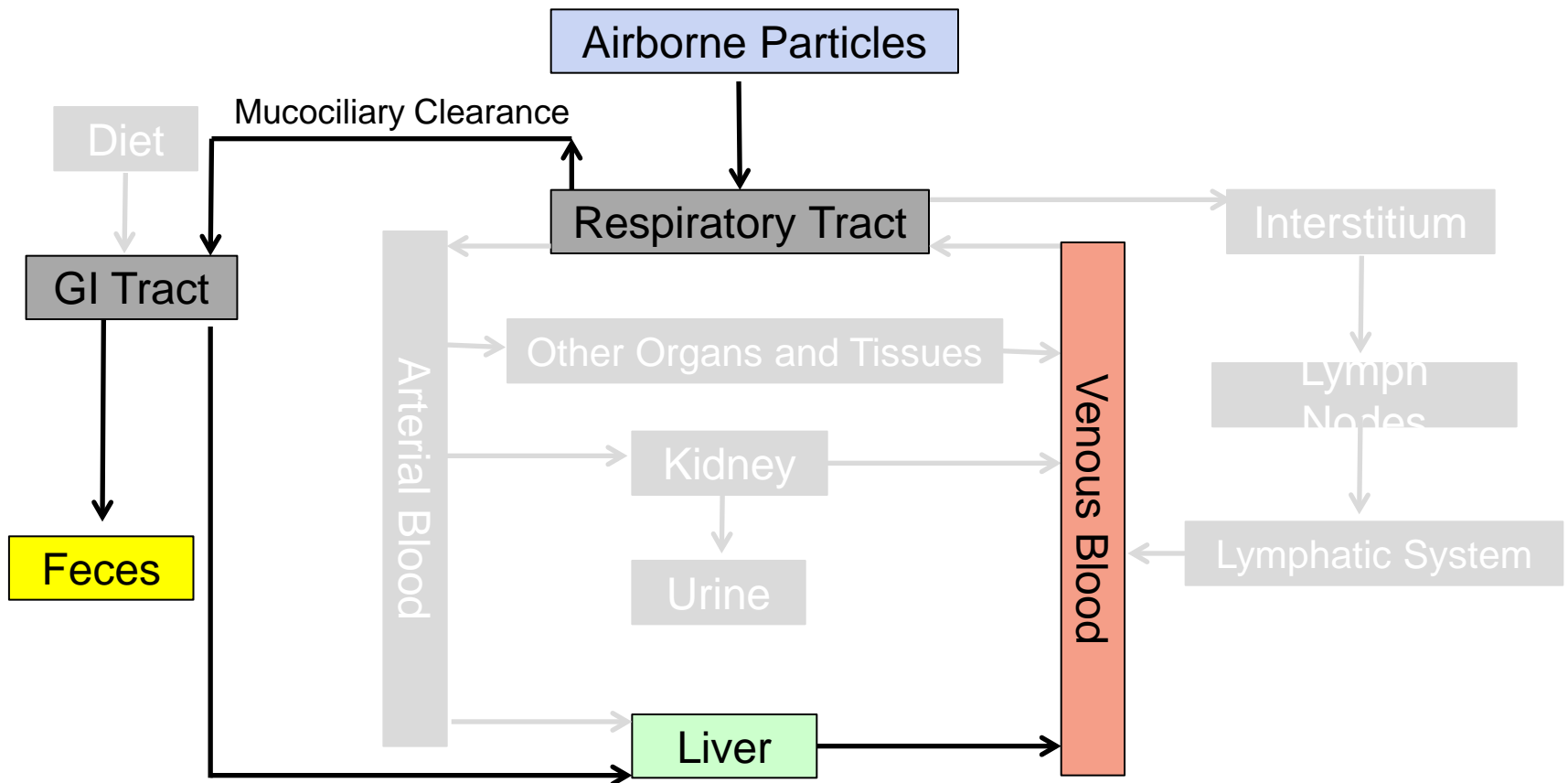
Soluble compounds/particles are rapidly absorbed





Inhalation Exposure

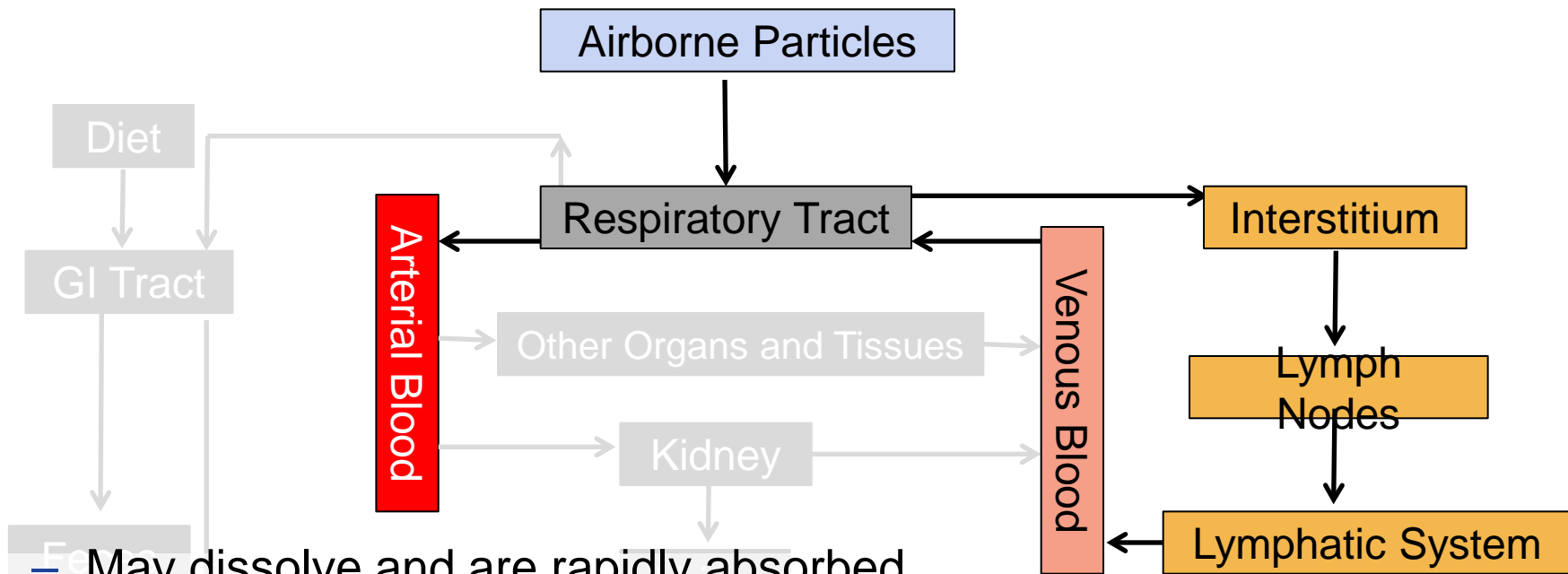
Large particles cleared by mucociliary action





Inhalation Exposure

Smaller particles reach the lower airways

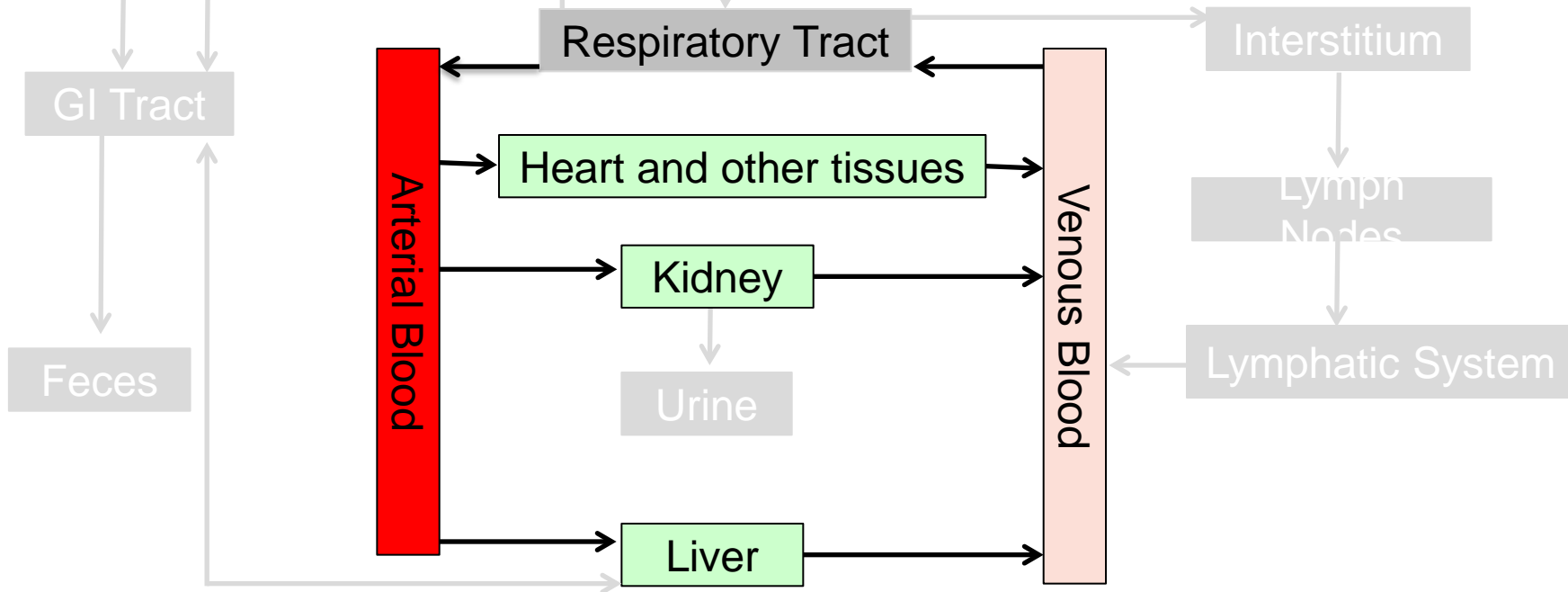


- May dissolve and are rapidly absorbed
- Phagocytized by alveolar macrophages
- Enter lung cells via endocytosis
- Nanoparticles may translocate directly to blood and lymph



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

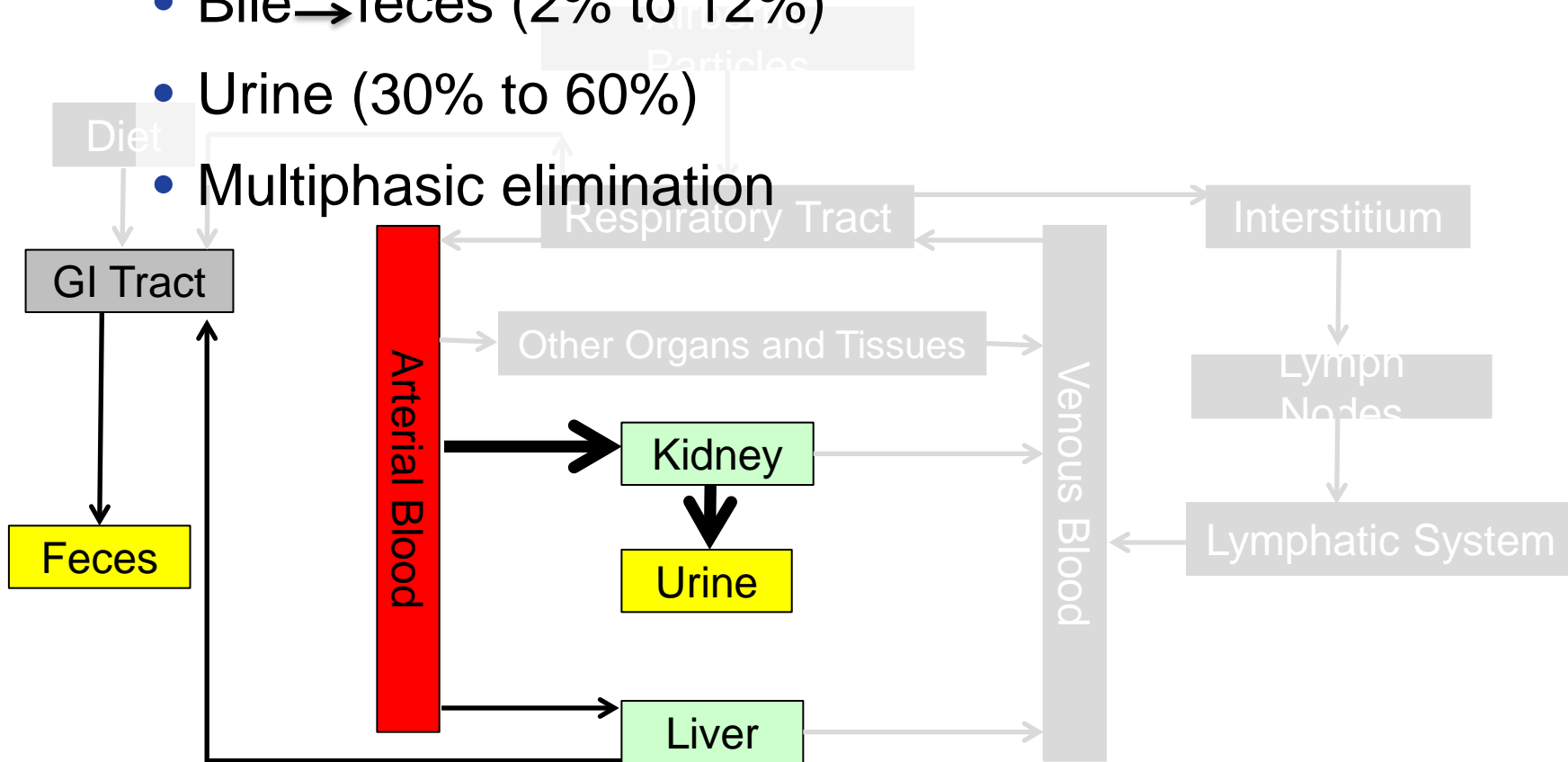




Cobalt Excretion

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

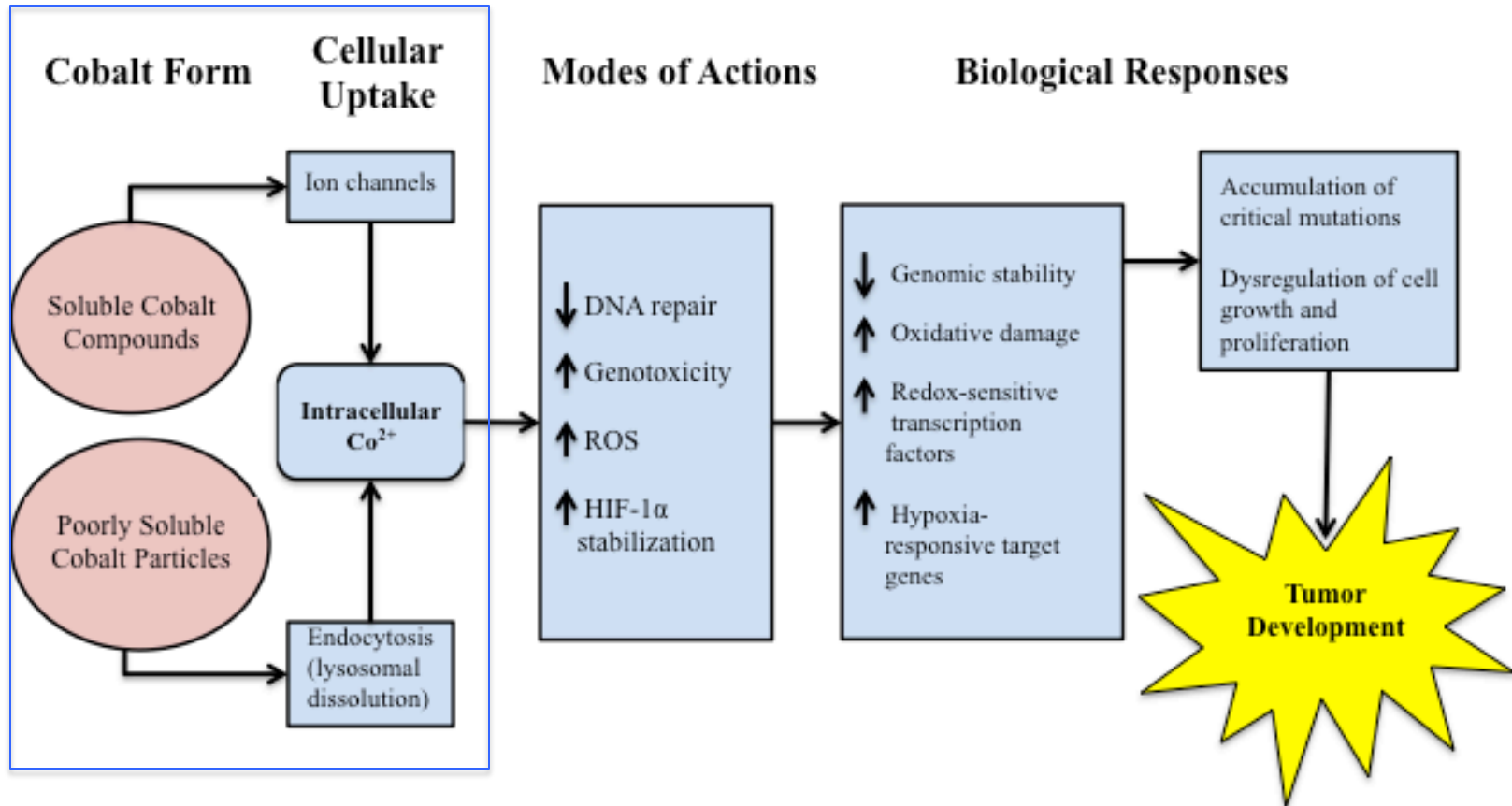
Disposition and Toxicokinetics

Mechanistic and Other Relevant Data

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



Cobalt and cobalt compounds affect similar pathways associated with carcinogenesis





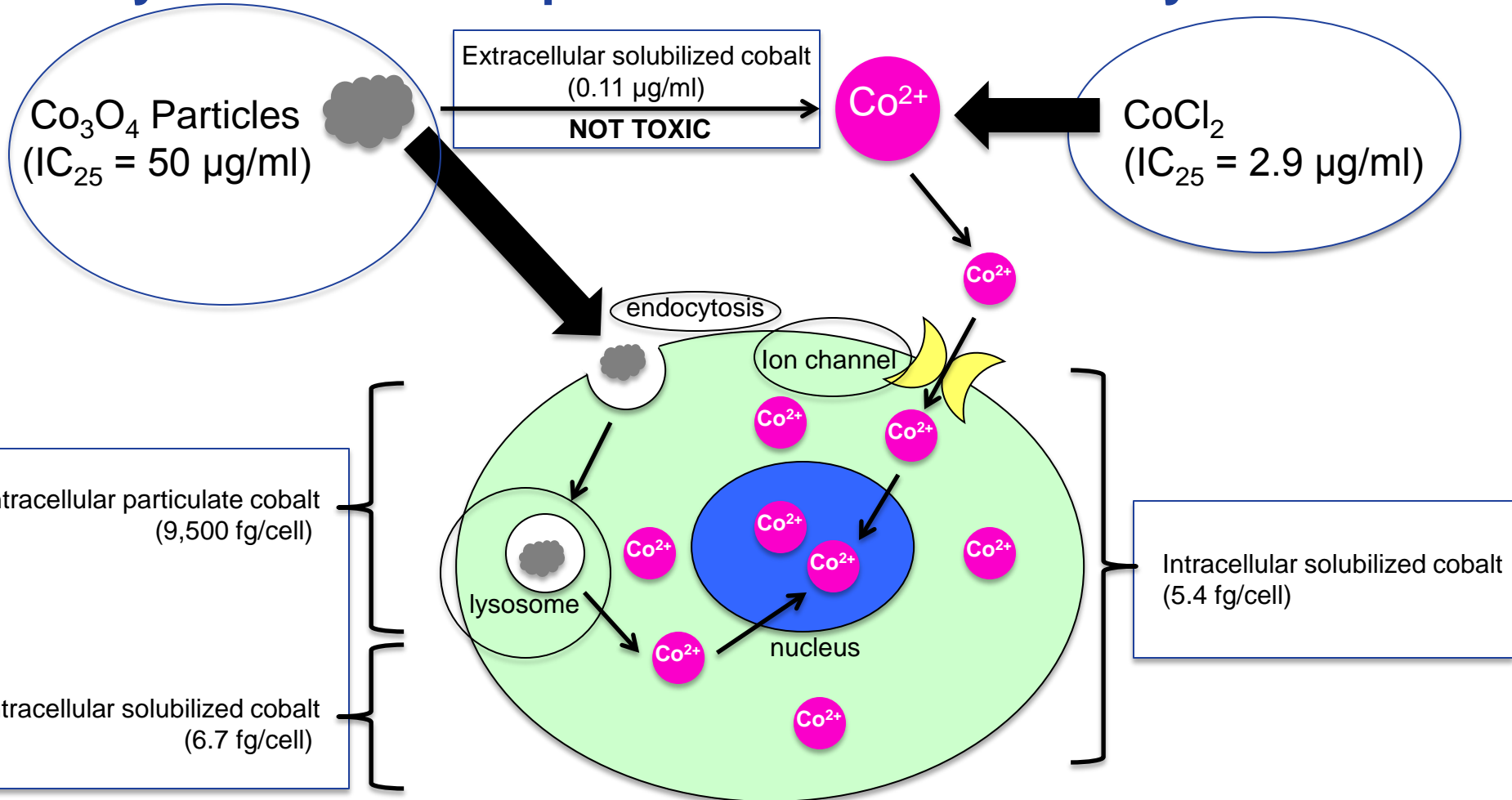
Cobalt particles and ions have similar biological effects *in vitro* and *in vivo*

- 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



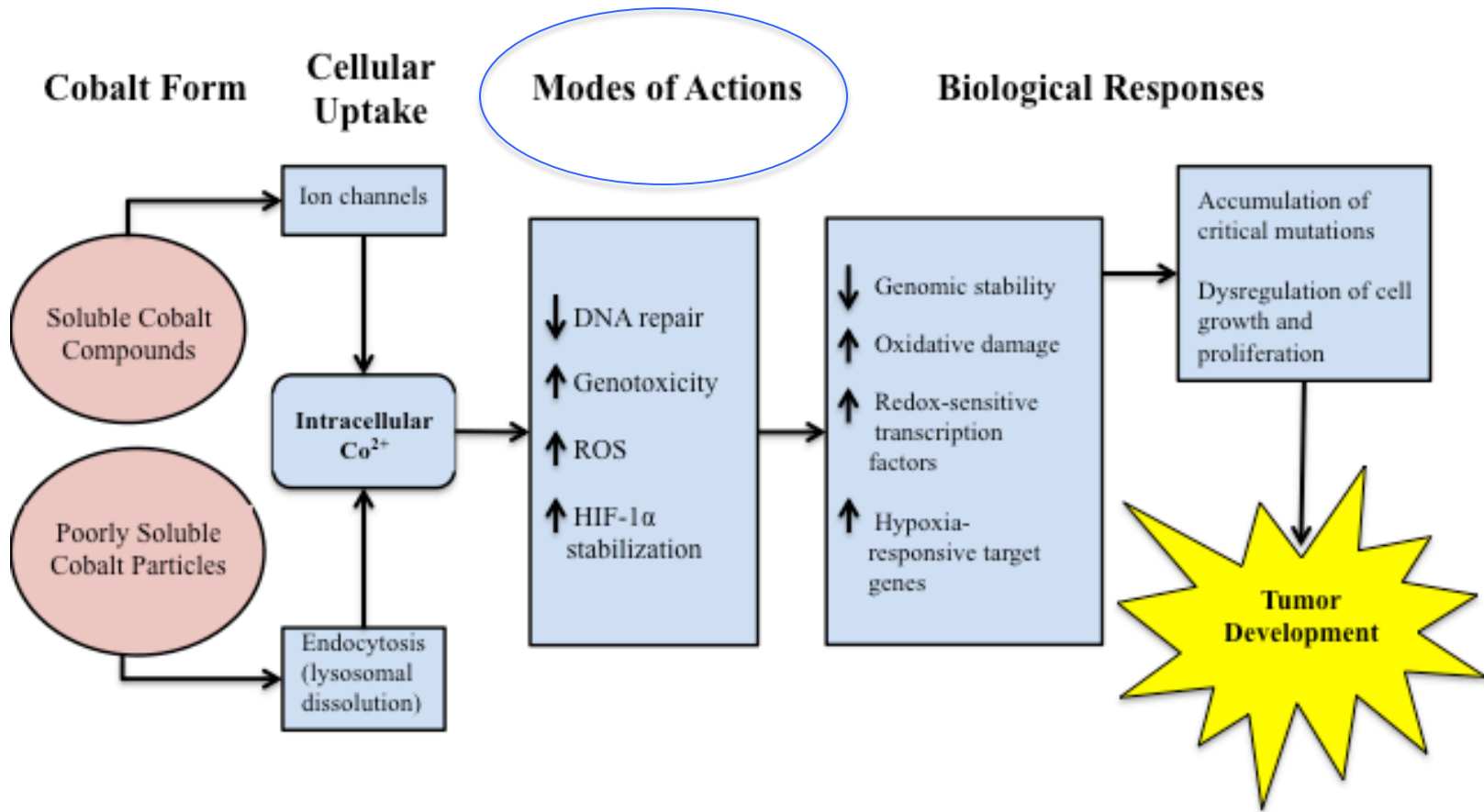
Cellular Uptake of Cobalt Ions and Particles

Poorly soluble cobalt particles dissolve in the lysosomes





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)

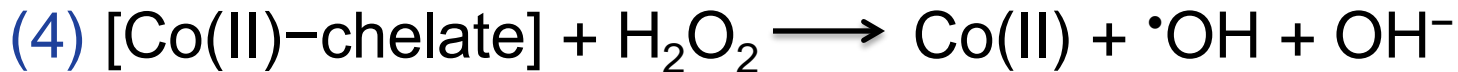
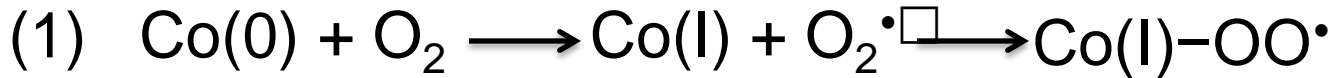


Cobalt inhibits DNA repair

- 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



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



ROS activate redox transcription factors

- NF- κ B
 - 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-1 α

- 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-1 α 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 than 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 ₃ O ₄
Bioaccessibility				
Lysosome*	+	+	+	+
Gastric	+	+	+	+
Cellular uptake	+	+	+	+
Cytotoxicity	+	+	+	+
ROS	+	ND	+	+
HIF-1 α stabilization	+	+	+	+
DNA repair inhibition	+	ND	+	ND
Genotoxicity <i>in vitro</i>	+	+	+	+
Genotoxicity <i>in vivo</i>	+	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



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- 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





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?



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.