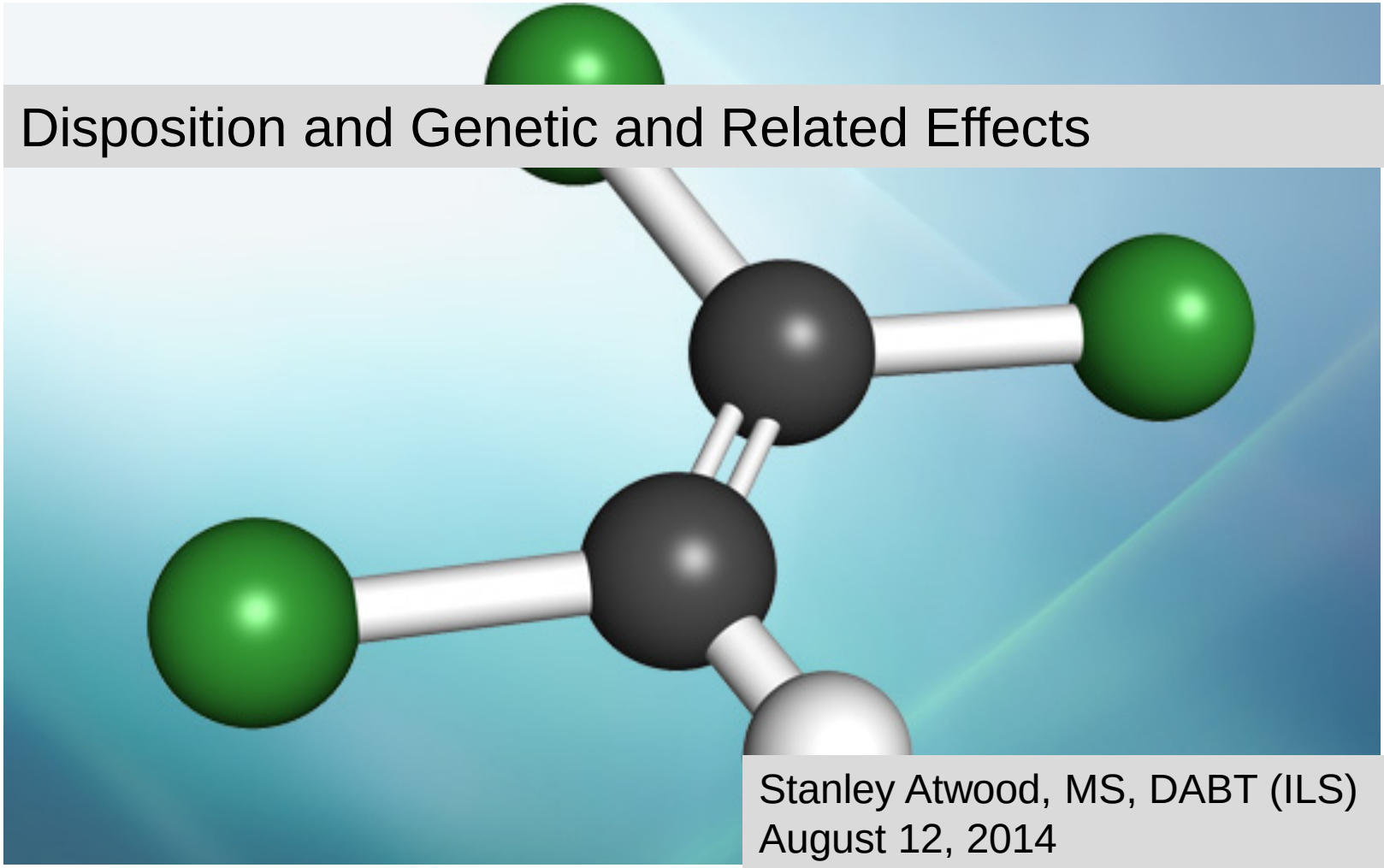
Disposition and Genetic and Related Effects



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August 12, 2014

TCE: Disposition and Genetic Effects

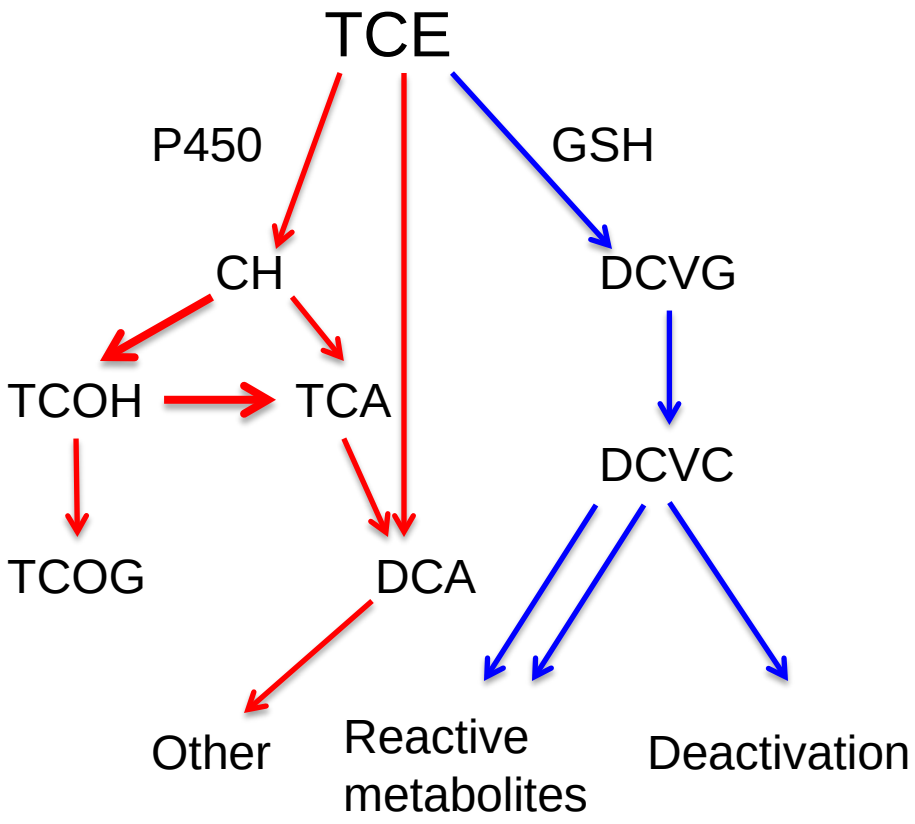
- Disposition
  - Absorption, distribution, excretion
  - Metabolism
- Genetic and related effects
  - TCE
  - Metabolites

Absorption, distribution and excretion

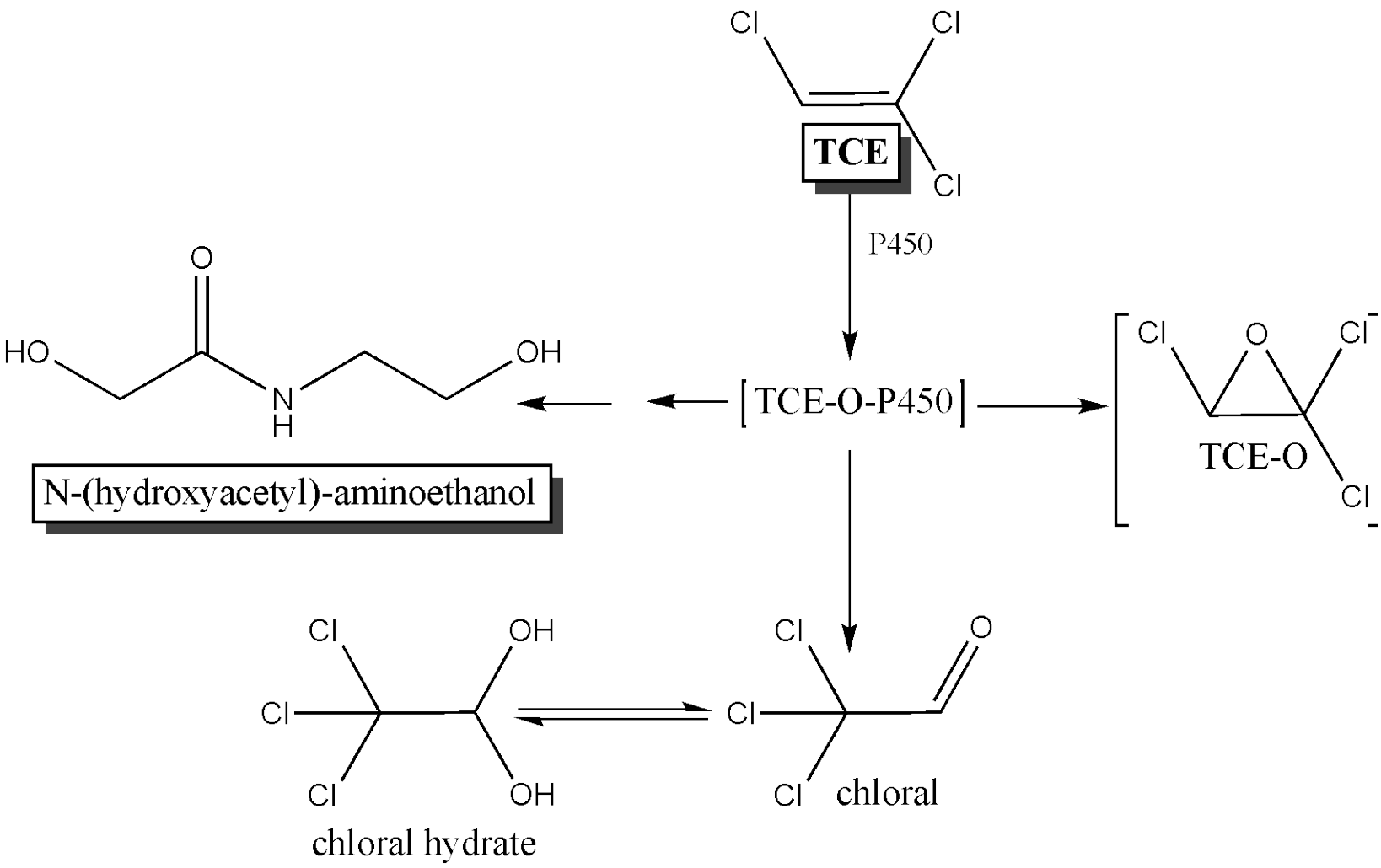
- Small lipophilic molecule
- Well absorbed from all routes
- Rapidly distributed to all tissues
  - Determined by blood:tissue partition coefficients
  - Highest concentrations in adipose tissue
- Excretion
  - Unchanged or CO_2 in exhaled breath
  - Metabolites in urine
  - Feces, sweat, saliva, milk

TCE metabolism is complex

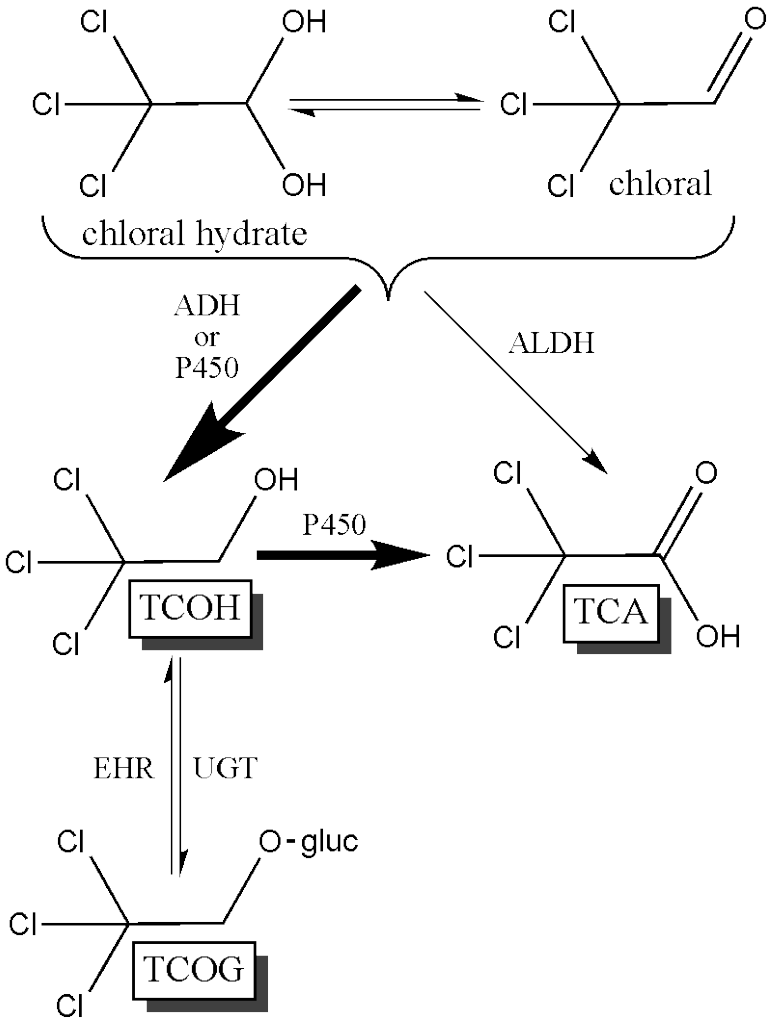
- Two key pathways
  - P450 (liver)
    - Dominant pathway
    - Several stable metabolites
  - GSH (liver/kidney)
    - Many reactive metabolites
    - One urinary metabolite
    - Flux uncertain (variable and can be altered)
  - Qualitatively similar in rodents and humans



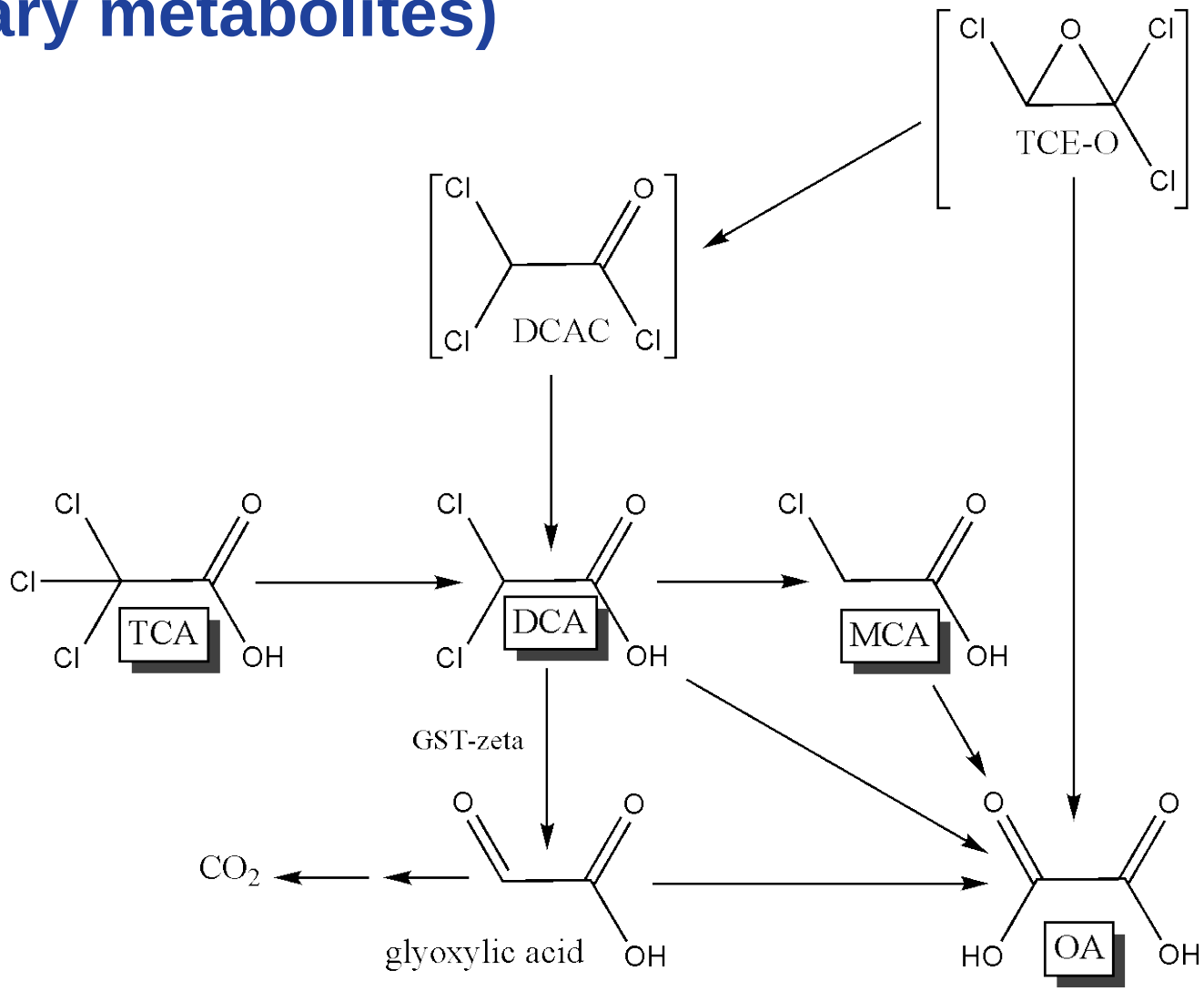
TCE metabolism: Oxidation (initial reactions)



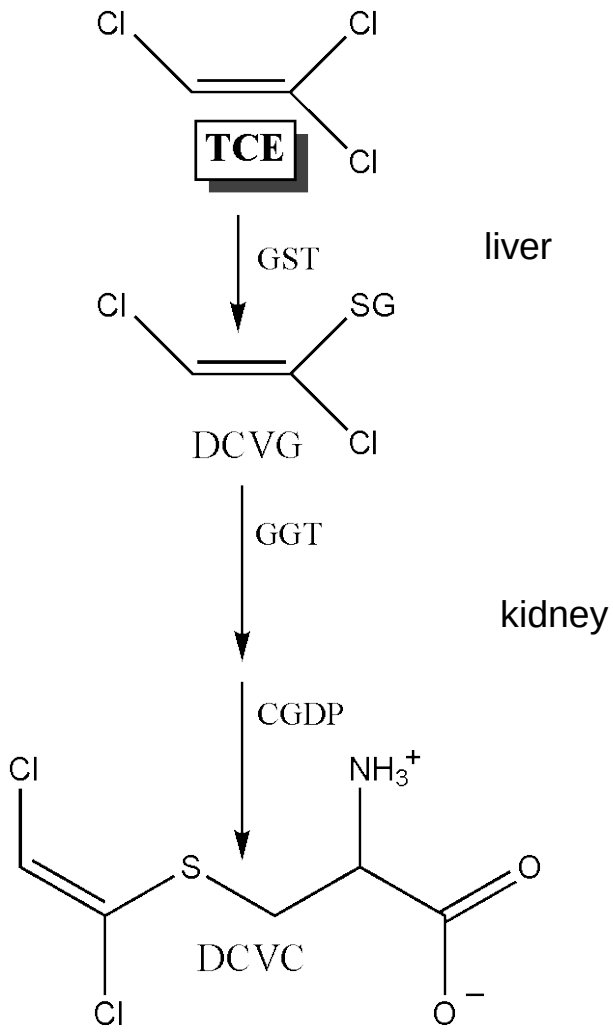
TCE metabolism: Chloral pathway (primary urinary metabolites)



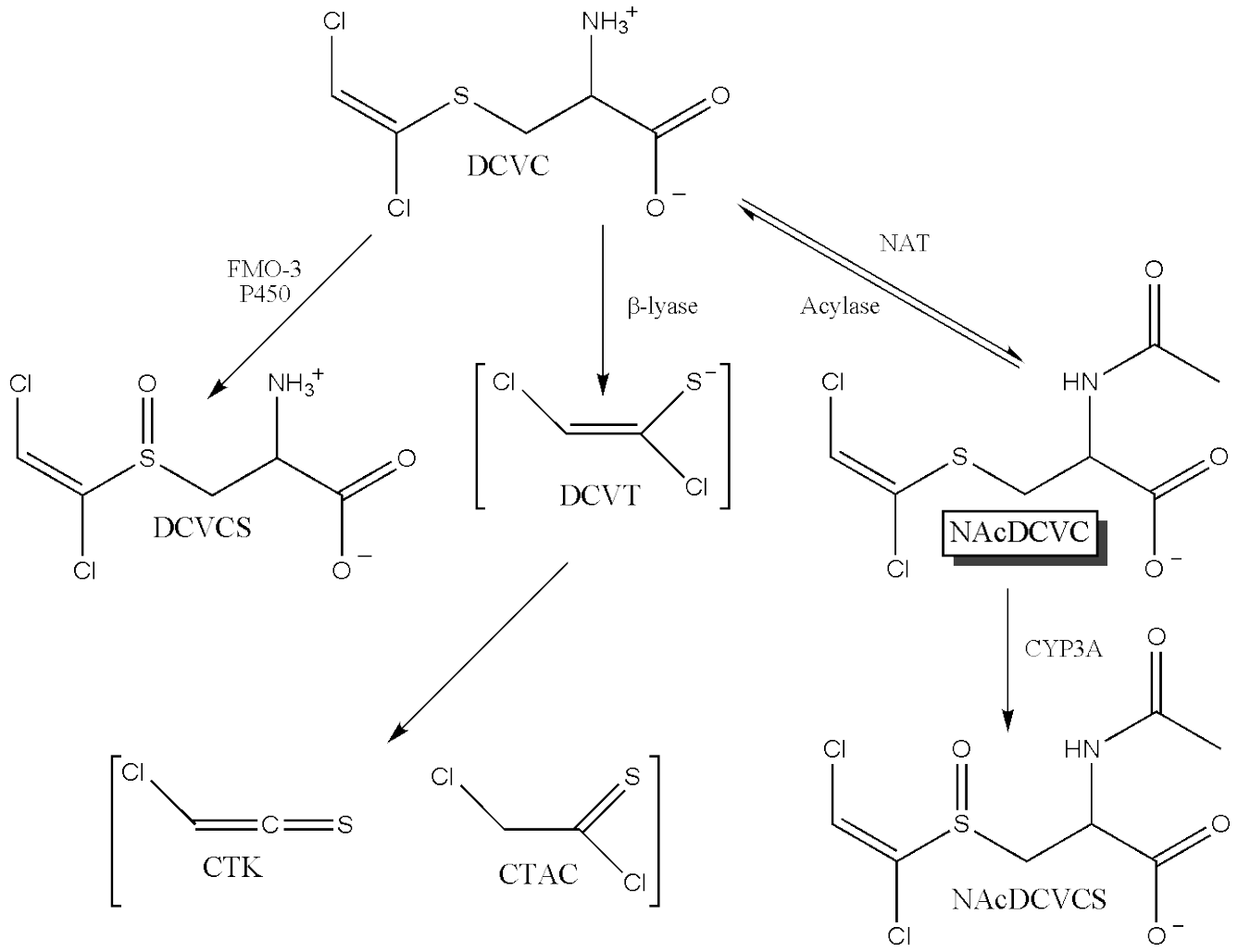
TCE Metabolism: TCE-oxide pathway (minor urinary metabolites)



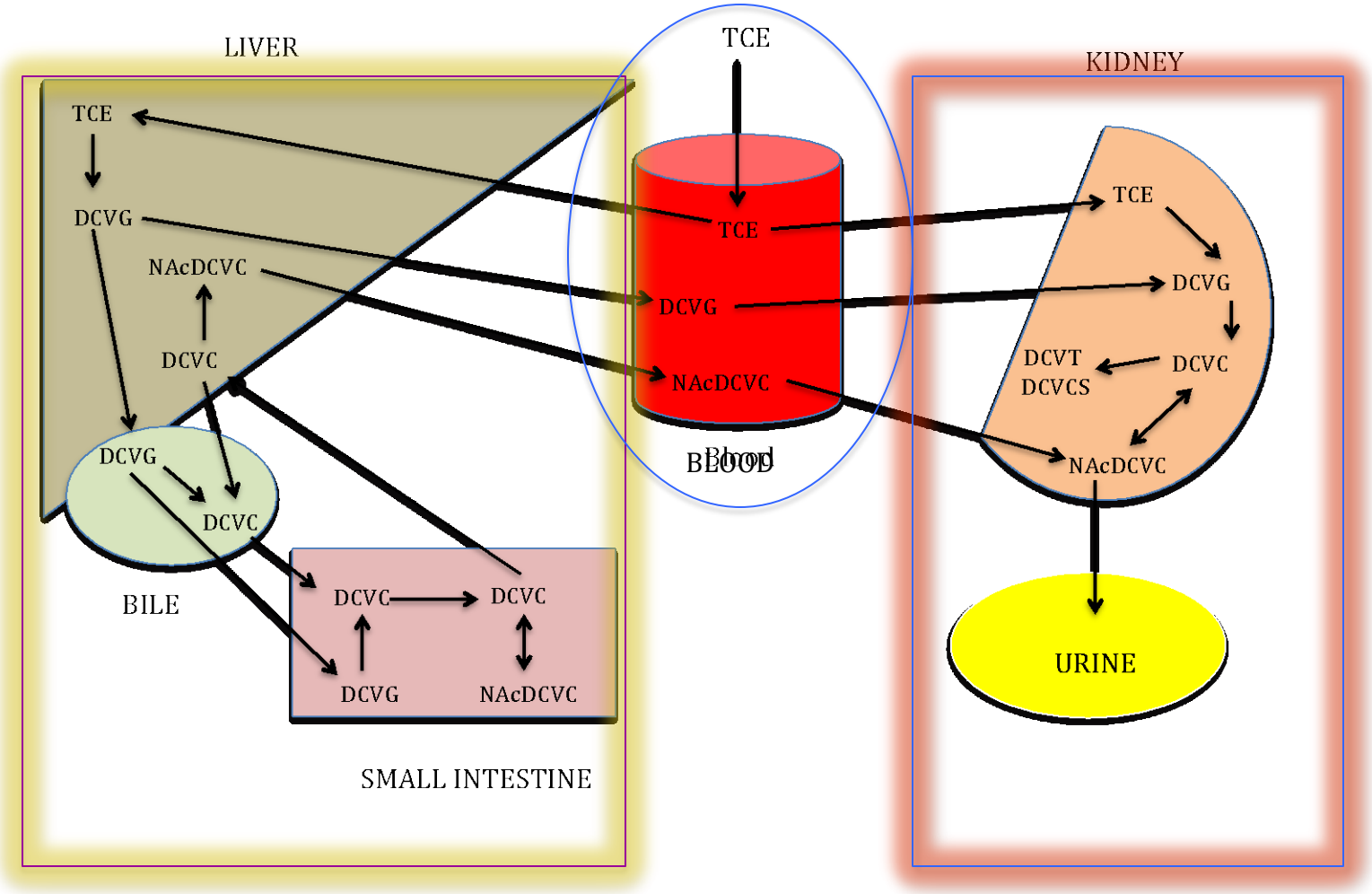
TCE Metabolism: GSH conjugation



TCE Metabolism: DCVC pathways (kidney)



TCE GSH Conjugates: Interorgan transport

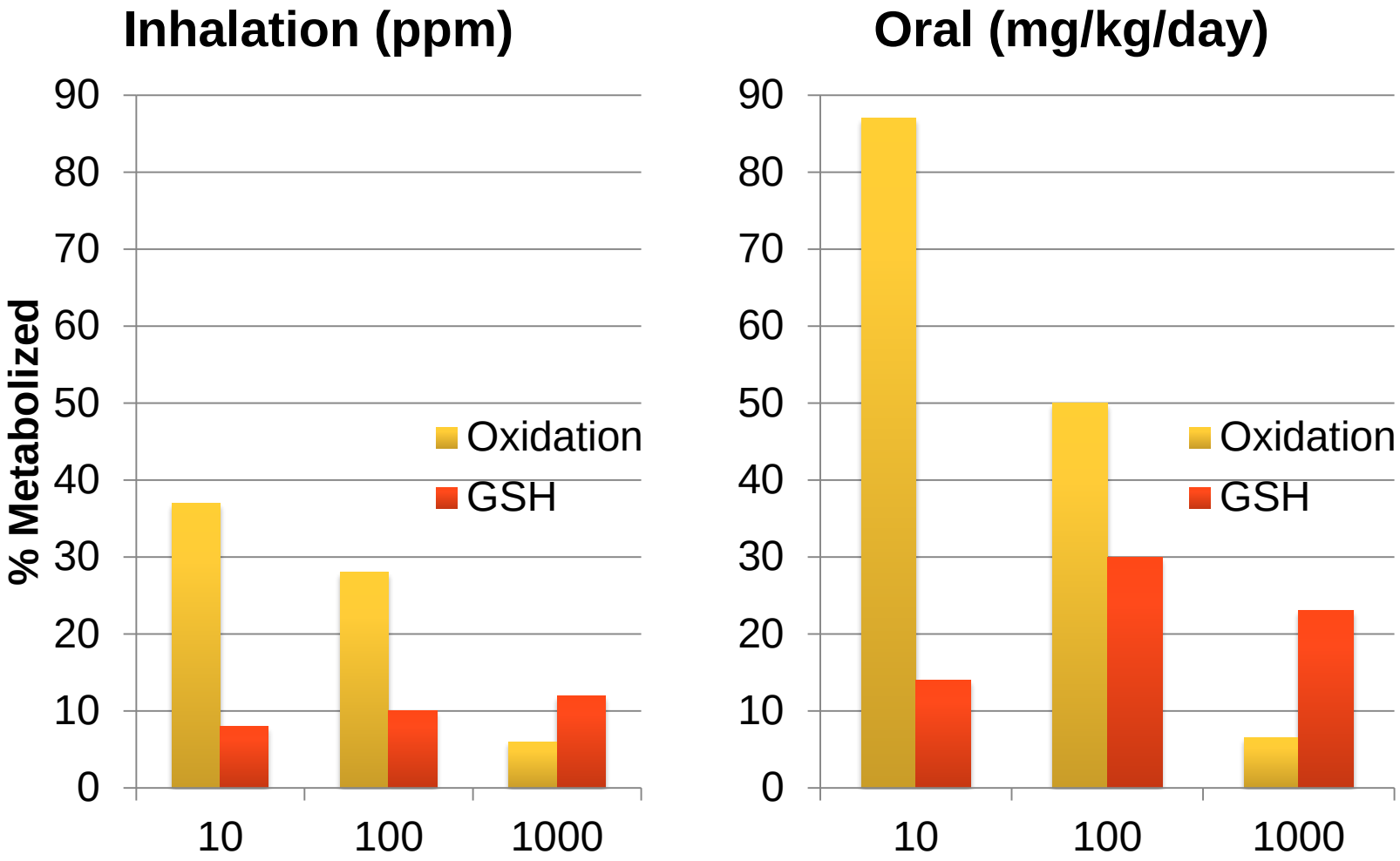


Adapted from Lash *et al.* 2014

TCE: GSH pathway is important

- Produces several reactive metabolites
- Flux estimates uncertain but may be higher than previously thought
  - Estimates of (TCA + TCOH)/NAcDCVC ≥ 1000:1 but NAcDCVC in urine is a small fraction of total flux
  - DCVG blood levels similar to TCA/TCOH in human volunteers
  - *In vitro* Km and Vmax values for oxidation and conjugation in human hepatocytes and subcellular fractions show overlap
- Genetic polymorphisms in GSTs/P450s/other
- Exposure to P450 inducers/inhibitors
  - Impact likely greater at high substrate concentrations

PBPK model predictions TCE oxidation vs. GSH conjugation - humans



TCE/metabolites cause genetic effects *in vitro*

Endpoint	TCE	CH	TCA	DCA	DCVG/DCVC
Mutations	(−)	+	(−)	(+)	+[b]
Aneuploidy	+	+	NT	−	NT
DNA strand breaks	+[a]	−	(−)	−	+[c]
UDS or DNA damage	(−)	+	−	+	+[c]
Clastogenic effects	+[a]	+	+	±	−
Cell transformation	±	+	NT	NT	+[c]
DNA/protein binding	+	−	NT	NT	+

− = negative, (−) = probably negative, ± = mixed results, (+) = probably positive, + = positive; NT = not tested

[a] Included strand breaks and MN in primary cultures of rat and human kidney cells and human HepG2 cells

[b] Effects increased with a kidney-derived activation system and diminished with β-lyase inhibitor

[c] Included effects in rodent kidney cells

TCE/metabolites cause genetic effects *in vivo*

Endpoint	TCE	CH	TCA	DCA	DCVG/DCVC
Mutations	–	NT	NT	+	NT
Aneuploidy	NT	±[a]	NT	NT	NT
DNA strand breaks	±[b]	±[c]	±[c,d]	(–)	±[e]
Clastogenic effects	(+)[f]	+[g]	+	(–)	NT
DNA/protein binding	±	–	+	NT	+

– = negative, (–) = probably negative, ± = mixed results, (+) = probably positive, + = positive; NT = not tested

[a] Positive in 2 of 4 studies in mouse blood or sperm

[b] Generally positive in liver in rats/mice, kidney in mice, inconsistent findings in rat kidney

[c] Positive in one study in liver (rats/mice) but not in another

[d] Also positive in human HepG2 cells

[e] Positive in rat proximal tubule cells (10 mg/kg at 2 hr); negative at 16 hr and at 1 mg/kg. Positive in male albino rabbit *in vivo* and in rabbit isolated kidney and proximal tubules (*ex vivo*).

[f] Included MN in rat kidney

[g] Included MN and SCE in peripheral blood lymphocytes of treated infant.

TCE Disposition and Genetic Effects: Summary

- Well absorbed from all routes and rapid distribution
- Highest tissue concentrations in fat
- Excreted unchanged in exhaled breath and as urinary metabolites
- Complex metabolism
  - Similar in humans and rodents
  - P450 pathway: TCA, TCOH, TCOG (liver)
  - GSH pathway: DCVC and other reactive metabolites (kidney)
- Genetic effects attributed to metabolites
  - Mutations
  - DNA and chromosome damage (MN)

TCE Disposition and Genetic Effects: Reviewer's questions/discussion

- Comment on whether the information on ADME and toxicokinetics is clear, technically correct, and objectively presented.
  - Identify any information that should be added or deleted.
- Comment on whether the genotoxicity data presented in the cancer evaluation component for TCE are clear, technically correct, and objectively presented.
  - Provide any scientific criticisms of the NTP's interpretation and application of the genotoxicity data from the cited studies for assessing effects of TCE.