Mouse Lung Carcinogens, Reactive Metabolites and Toxicity

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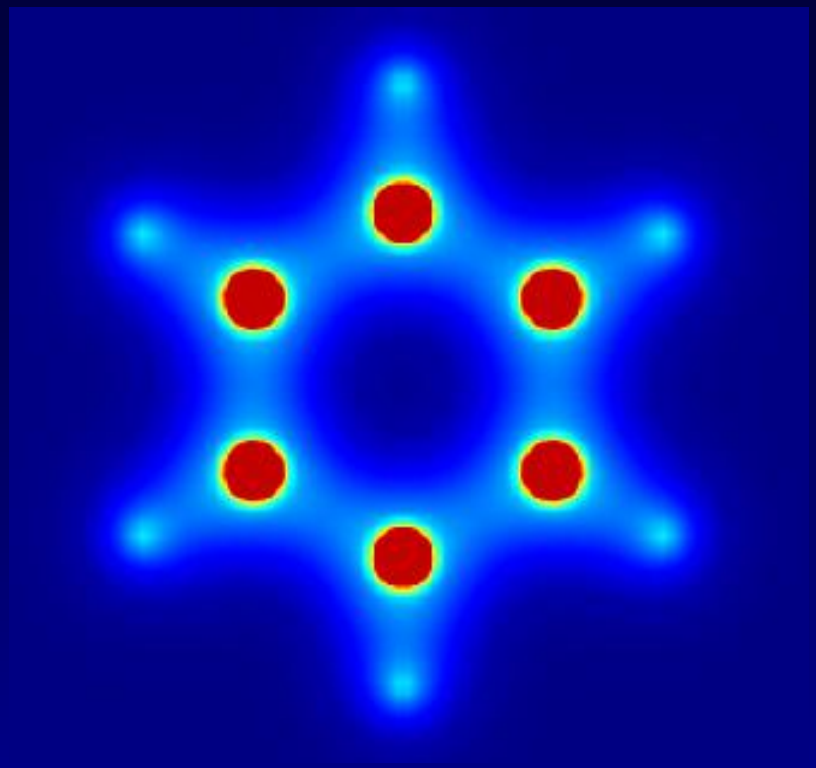
Mouse Lung Carcinogens – Selected NTP Results

Chemical	NTP Lung tumors	NTP conclusion	NTP non-cancer lung pathology
Benzene (gavage)	Significant increases in alveolar/bronchiolar adenoma or carcinoma seen in both male and female mice	Clear evidence (included all tumor sites)	Increases in hyperplasia seen in male and female mice
Ethylbenzene (inhalation)	A significant increase in alveolar/bronchiolar adenoma or carcinoma was seen at the high dose in male mice; increase within historical control range	Some evidence	Increased alveolar epithelial metaplasia seen in 2 yr study; no effects reported in 13 week study
Naphthalene (inhalation)	A significant increase in alveolar/bronchiolar adenoma (and 1 carcinoma) was seen in female mice	Some evidence	Increases in chronic inflammation were seen in both the male and female mice
Styrene (gavage)	A significant increase in lung adenomas and carcinomas combined was seen in male mice	Suggestive evidence; not convincing evidence	No significant changes reported.

Mouse Carcinogen Toxicity & Metabolism

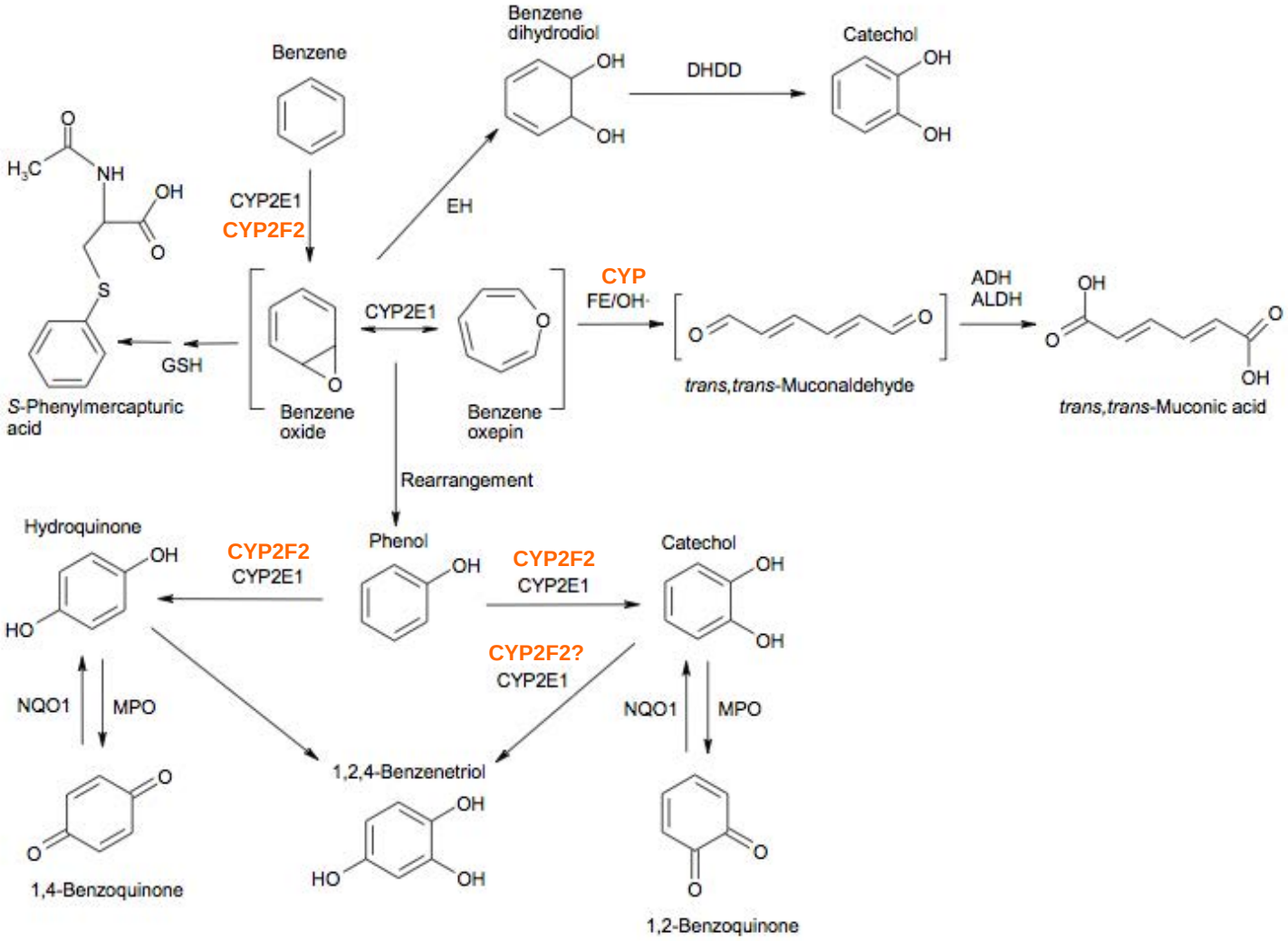
Chemical	Other non-NTP studies	Lung metabolism
Benzene	Slight lung toxicity reported in one study.	Metabolism by Cyp2F2 predominates in mouse lung
Ethylbenzene	Increases in DNA synthesis and decreases in metabolic enzymes reported in lungs in short-term studies	Metabolism by both Cyp2E1 and Cyp2F2 in lungs. Enhanced formation of ring hydroxylation products in vitro (at very low levels); no substantial evidence for their formation in vivo
Naphthalene	Naphthalene has been seen to damage the mouse lung in multiple studies; Selective damage to the Clara cells seen, particularly in the distal bronchioles.	Higher rate of metabolism in mouse. Metabolism by Cyp2F predominates in mouse lung (distal bronchioles), results in a toxic 1R,2S-epoxide, and correlates with Clara cell toxicity.
Styrene	Lung tumors and hyperplasia seen in mice in other studies and by inhalation. Cytotoxicity and cell proliferation in the lung (esp. Clara cells) seen in short-term studies.	Metabolism by Cyp2F2 predominates in mouse lung. Metabolites of 4-vinylphenol implicated in lung toxicity.



Benzene

- Important industrial chemical and environmental contaminant
- Bone marrow toxicant and leukemia-inducing agent
- Lung carcinogen in mice. There are several reports of it being associated with lung cancer in humans
- Multiple metabolic pathways, and most likely, multiple mechanisms of action. The critical ones remain elusive.

Figure 3-3. Metabolic Pathways for Benzene



ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; CYP2E1 = cytochrome P-450 2E1; DHDD = dihydrodiol dehydrogenase; EH = epoxide hydrolase; GSH = glutathione; MPO = myeloperoxidase; NQ01 = NAD(P)H:quinone oxidoreductase

Source: adapted from Nebert et al. 2002; Ross 2000

ATSDR, 2007

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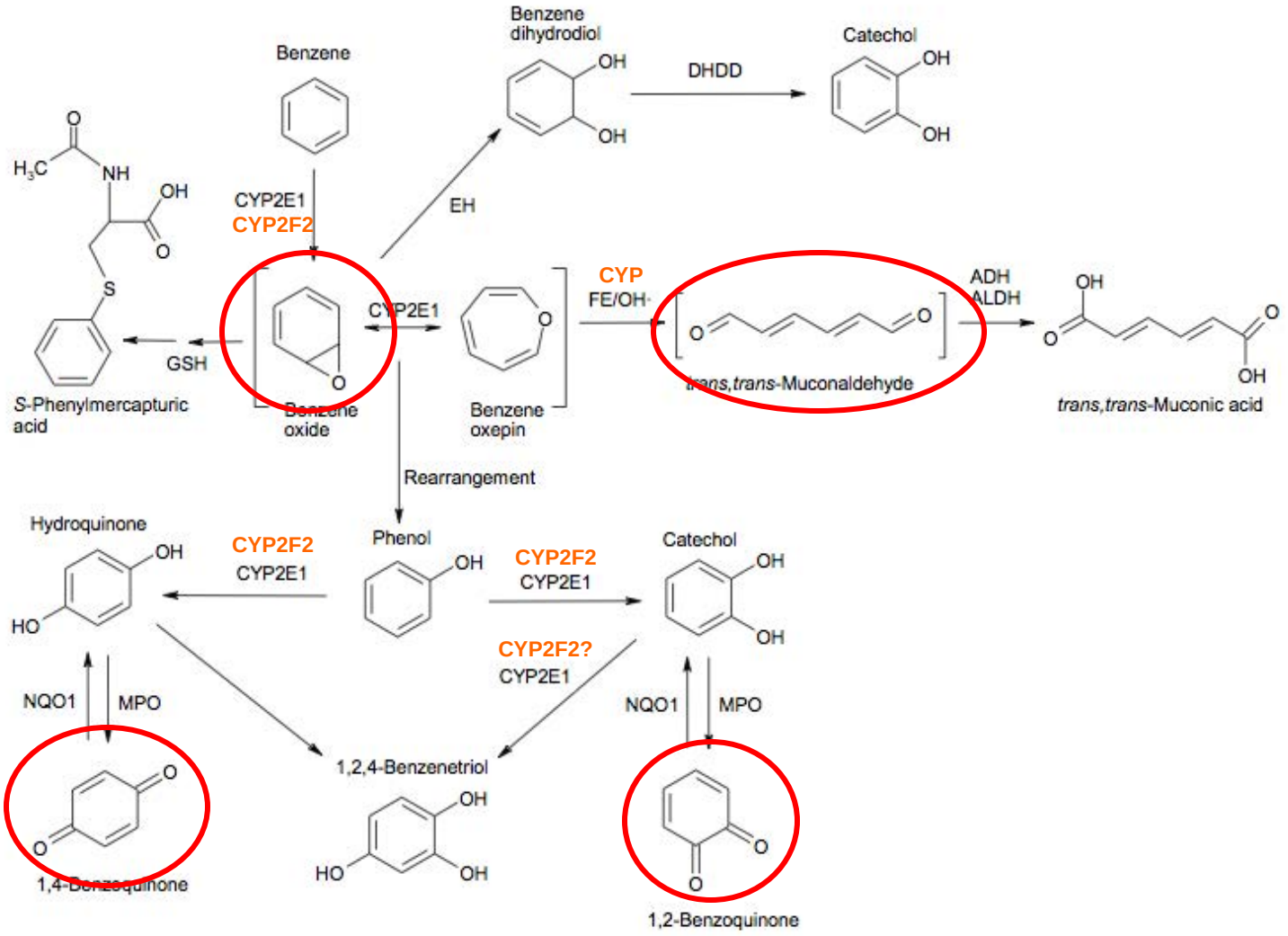

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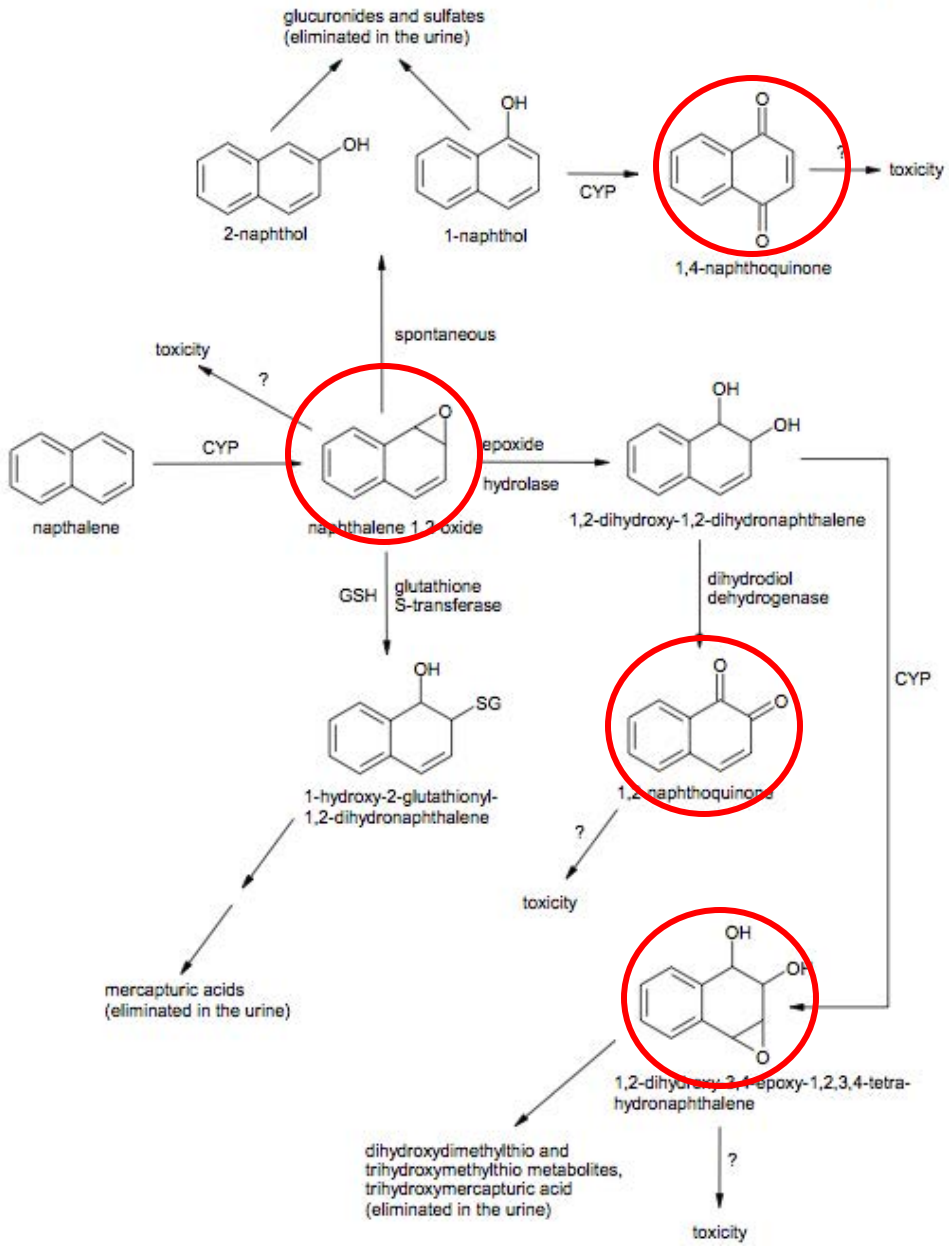
Others

- Hydroxybenzoquinone
- Benzoquinone epoxide
- GSH conjugated BQ
- Biphenols and their oxidation products
- Diol epoxides
- Semiquinones and other radicals
- Reactive oxygen and nitrogen species
- Nitro-derivatives

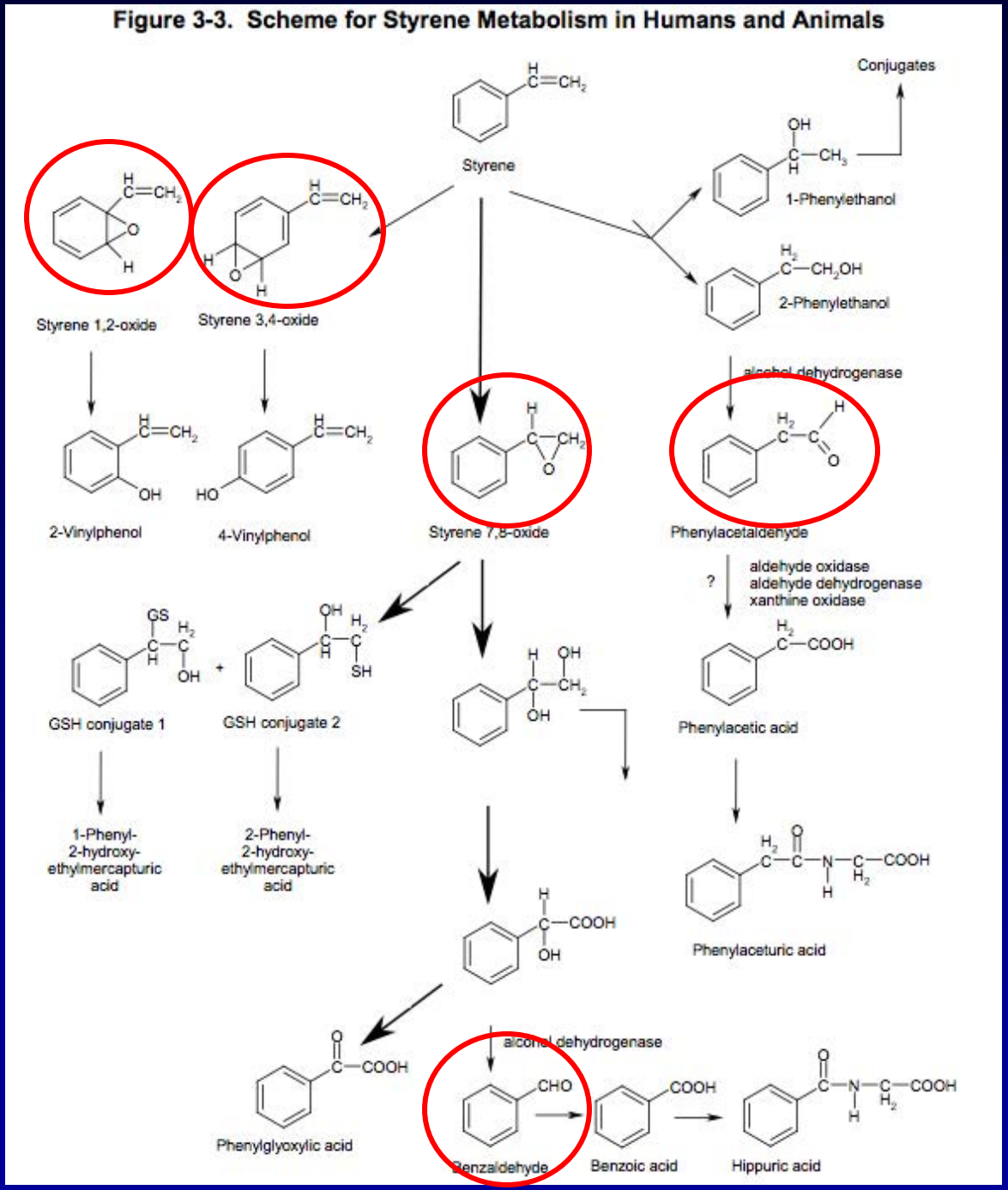
ATSDR, 2007

Figure 3-3. Scheme for Naphthalene Metabolism and Formation of Multiple Reactive Metabolites, That May Be Involved in Naphthalene Toxicity*



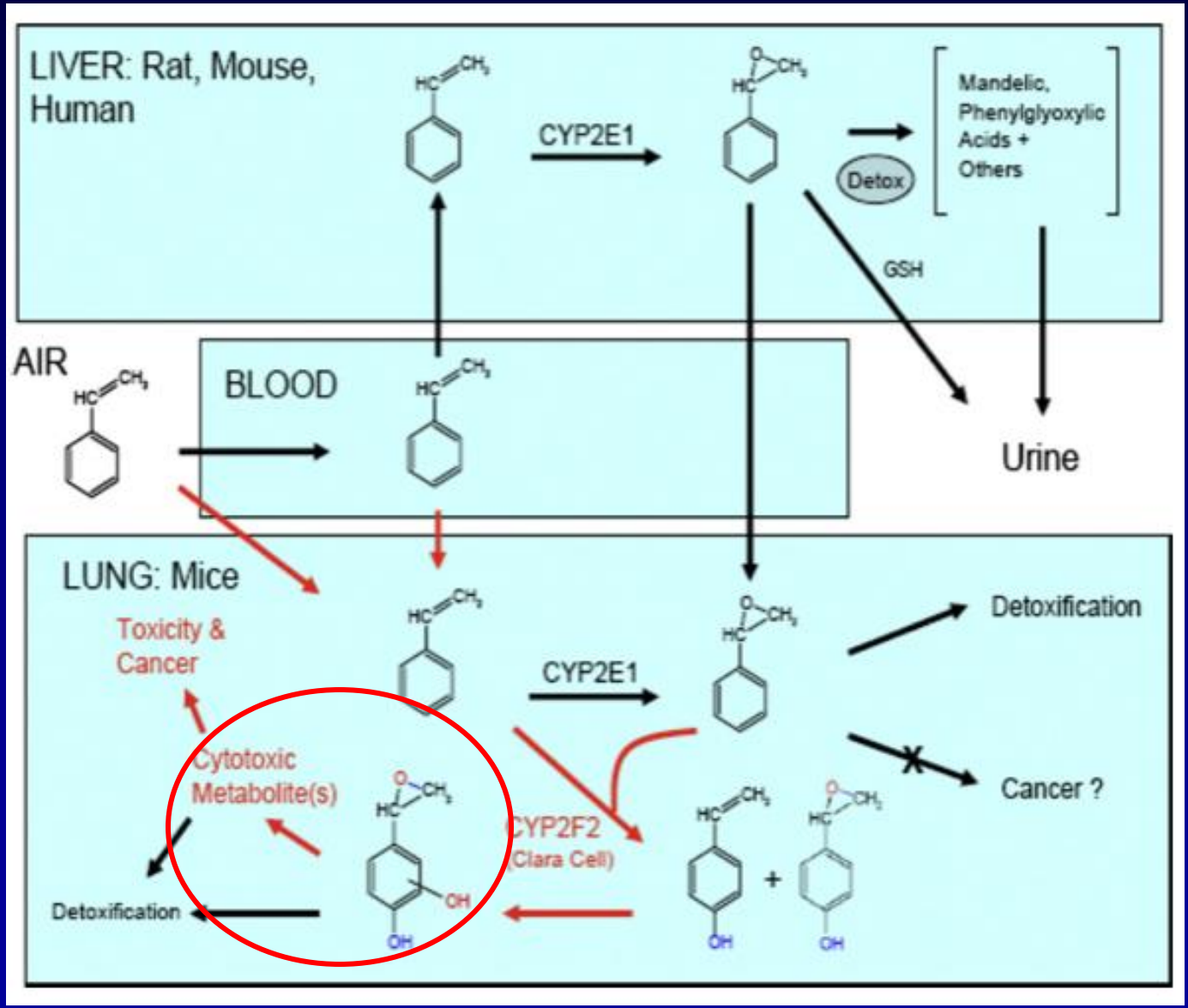
ATSDR, 2005

Figure 3-3. Scheme for Styrene Metabolism in Humans and Animals



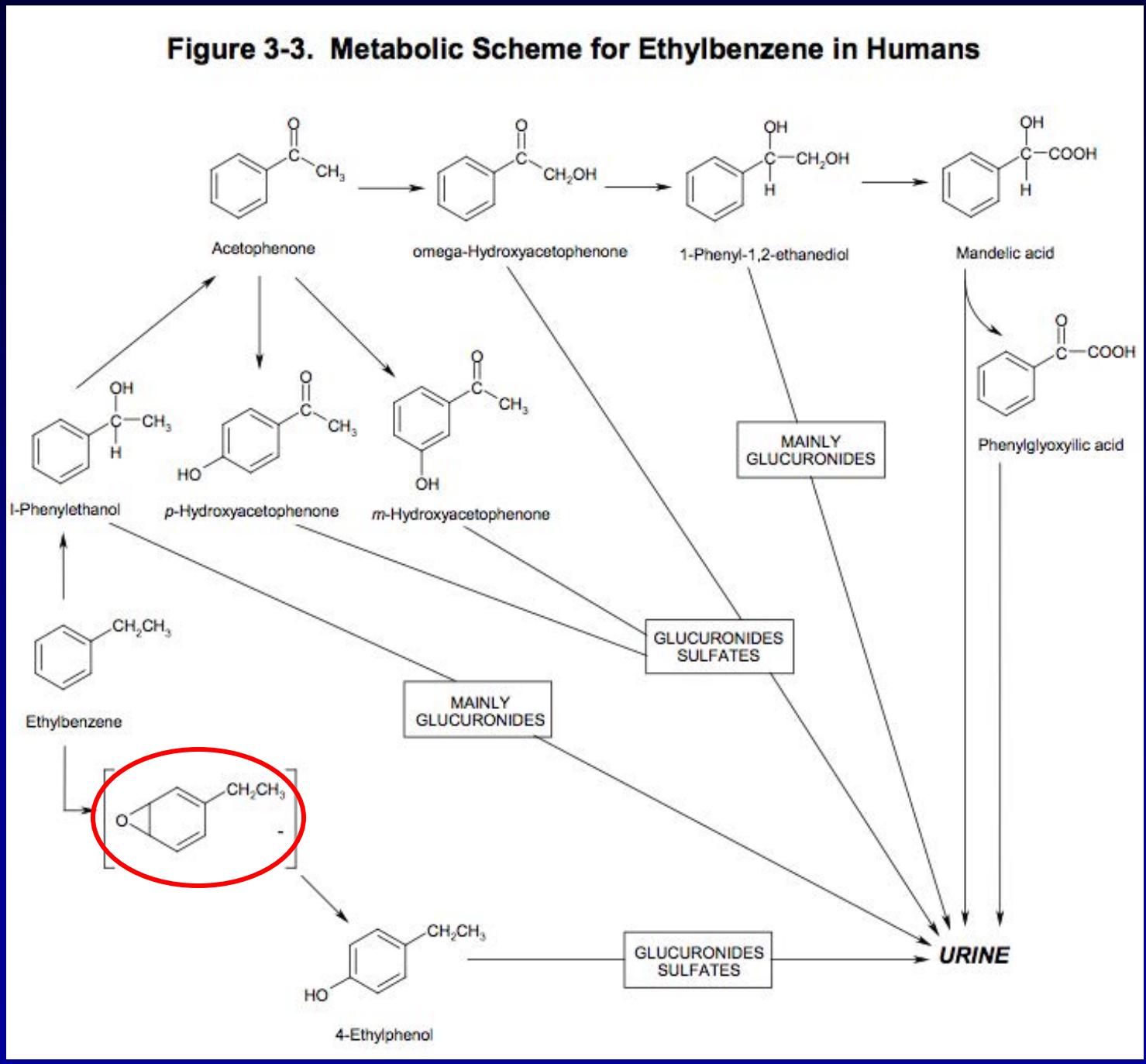
ATSDR, 2010

Postulated Styrene Metabolism and MOA



Cruzan et al. (2012)

Figure 3-3. Metabolic Scheme for Ethylbenzene in Humans



ATSDR, 2010

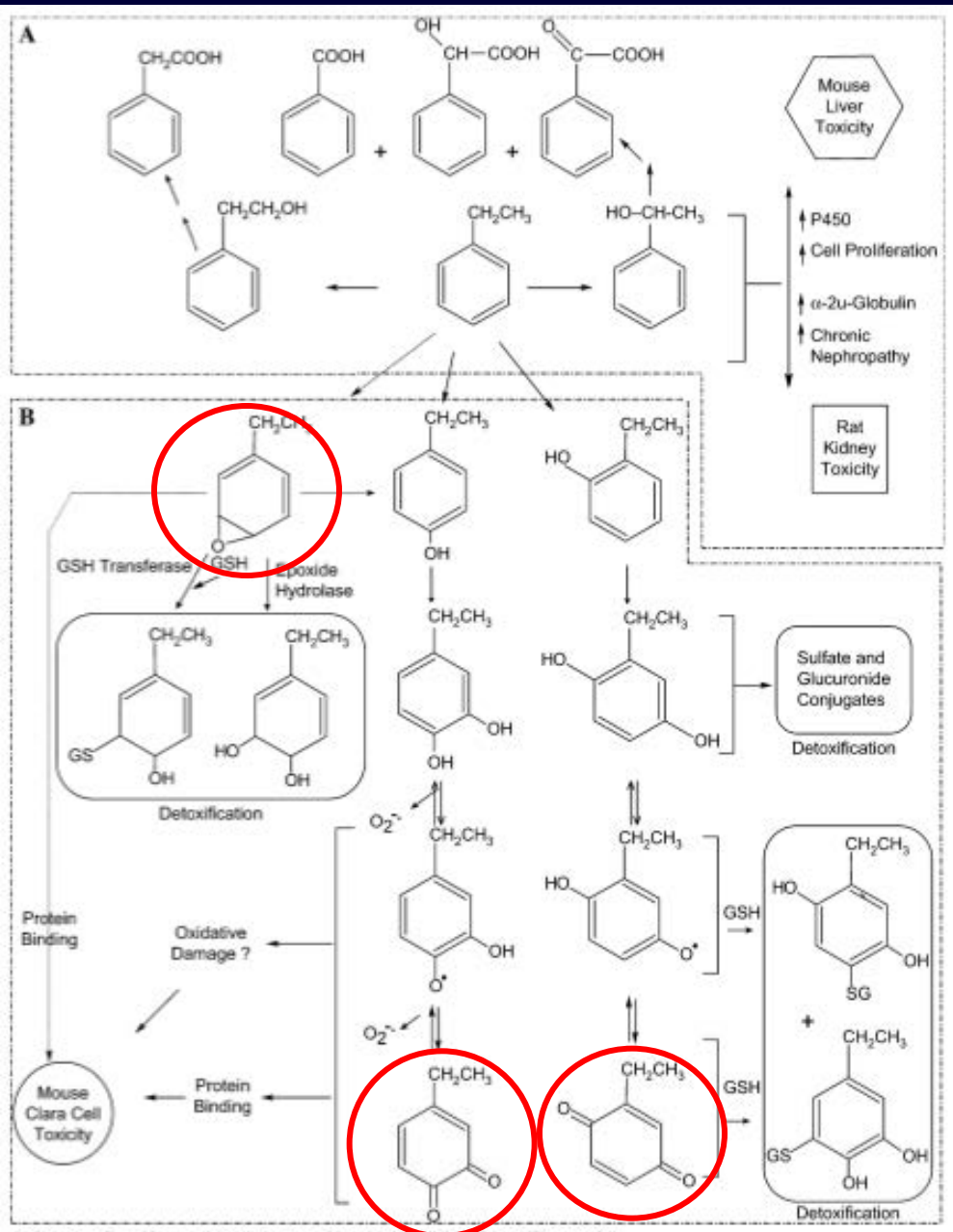
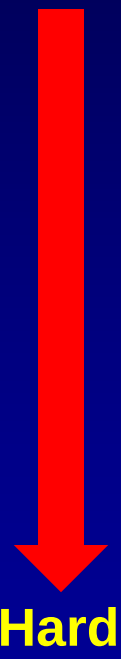


FIG. 1. Proposed metabolism of EB in the liver and lung causing toxicities. A) Rat and mouse liver-predominant metabolism; B) mouse lung pre-dominant metabolism.

Saghir et al. 2009

Examples of Hard and Soft Electrophiles and Nucleophiles



Electrophiles

- Quinones, aldehydes, polarized double bonds
- Epoxides, strained ring lactones, alkyl sulfates, alkyl halides
- Arylcarbonium ions
- Benzylic carbonium ions, nitrenium ions
- Alkyl carbonium ions

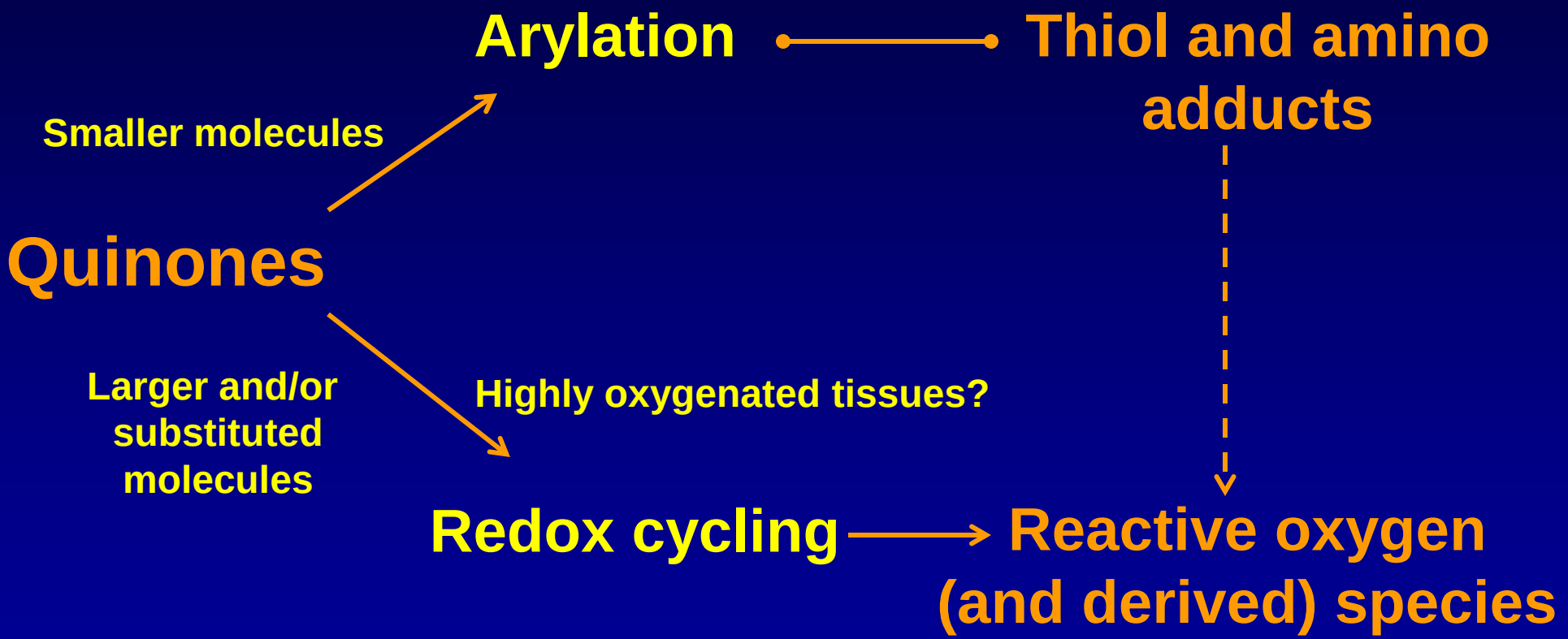
Nucleophiles

- Thiol groups of cysteine residues
- Sulfur of methionine
- 1° and 2° amino groups of proteins
- Amino groups in purine nucleobases
- Oxygen atoms of nucleotides
- Phosphate oxygens of DNA & RNA

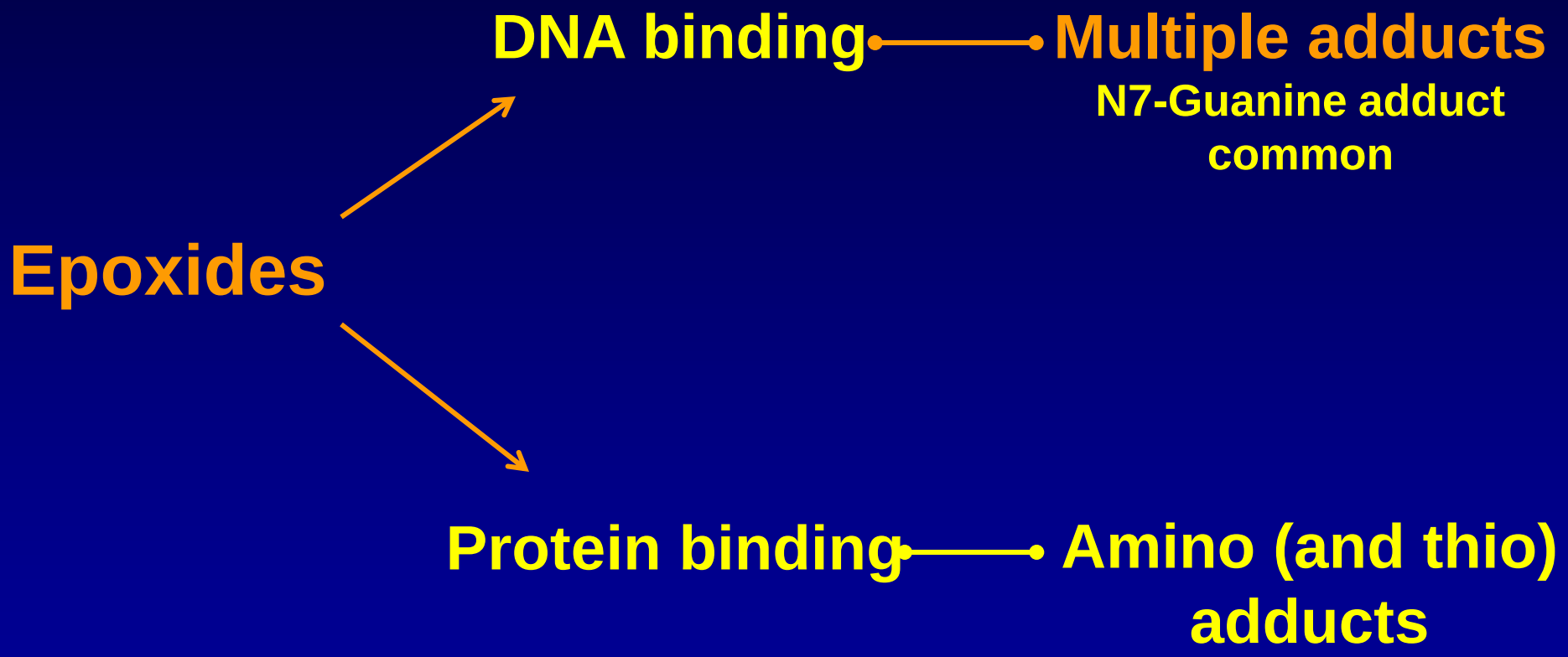
Note: The preferred target can be influenced by the tissue microenvironment.

Modified from Coles (1984-85)

Common Reactions of Quinones



Common Reactions of Epoxides



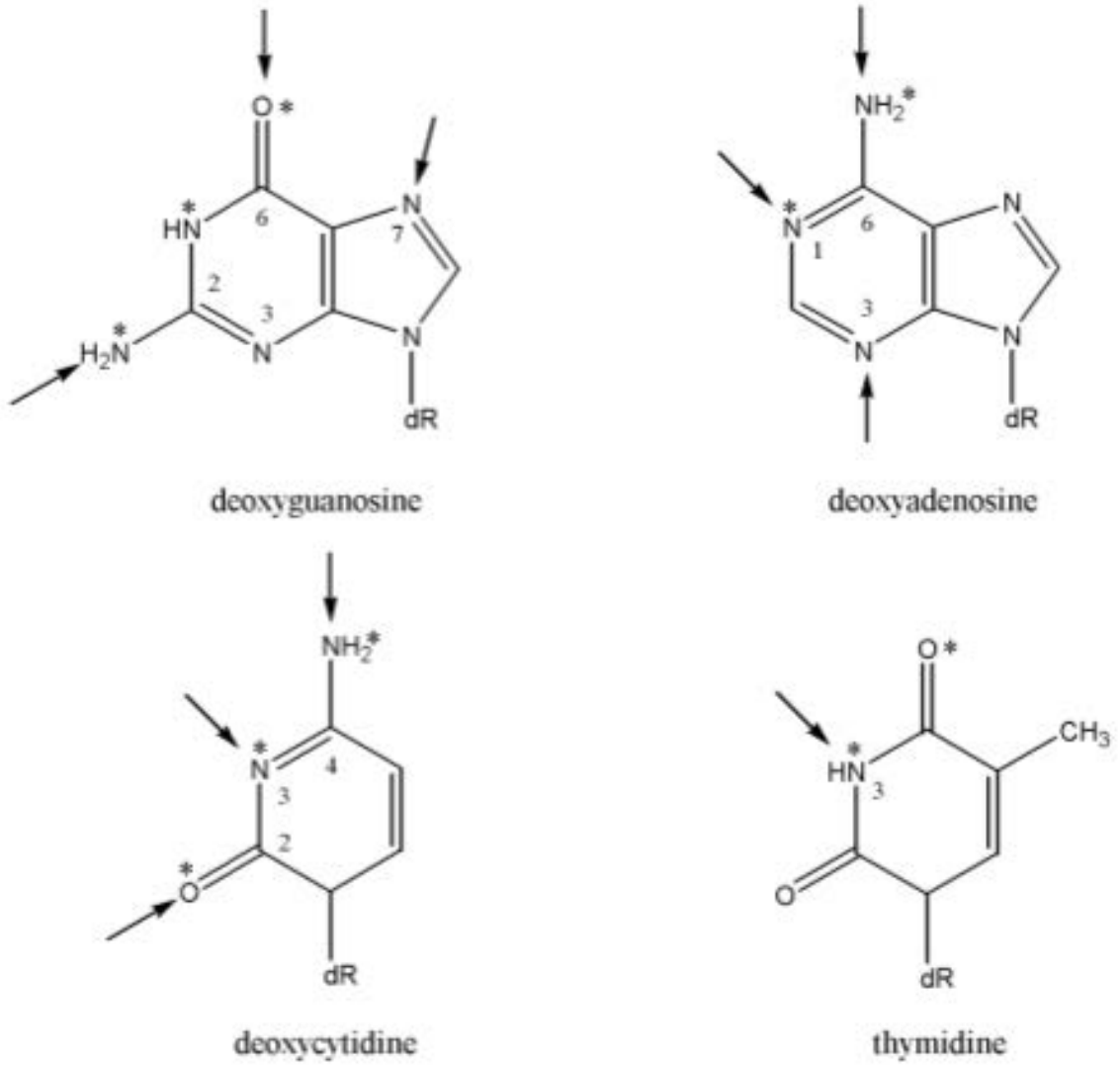
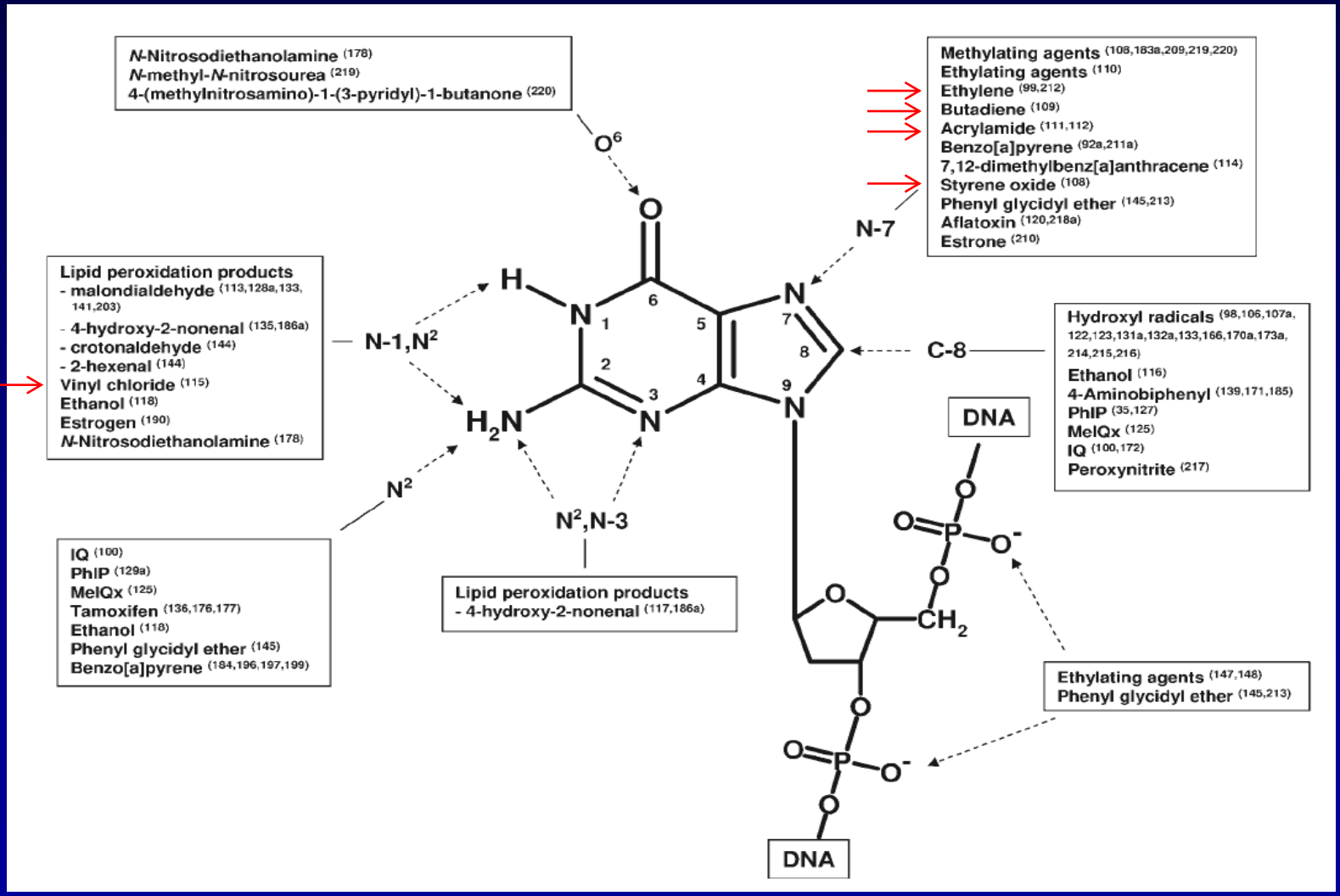


Figure 5-3. Styrene-7,8-oxide binding sites in DNA (from Vodicka *et al.* 2002a)
Styrene-7,8-oxide-binding sites are indicated by arrows; base-pairing sites in DNA are labeled with asterisks.

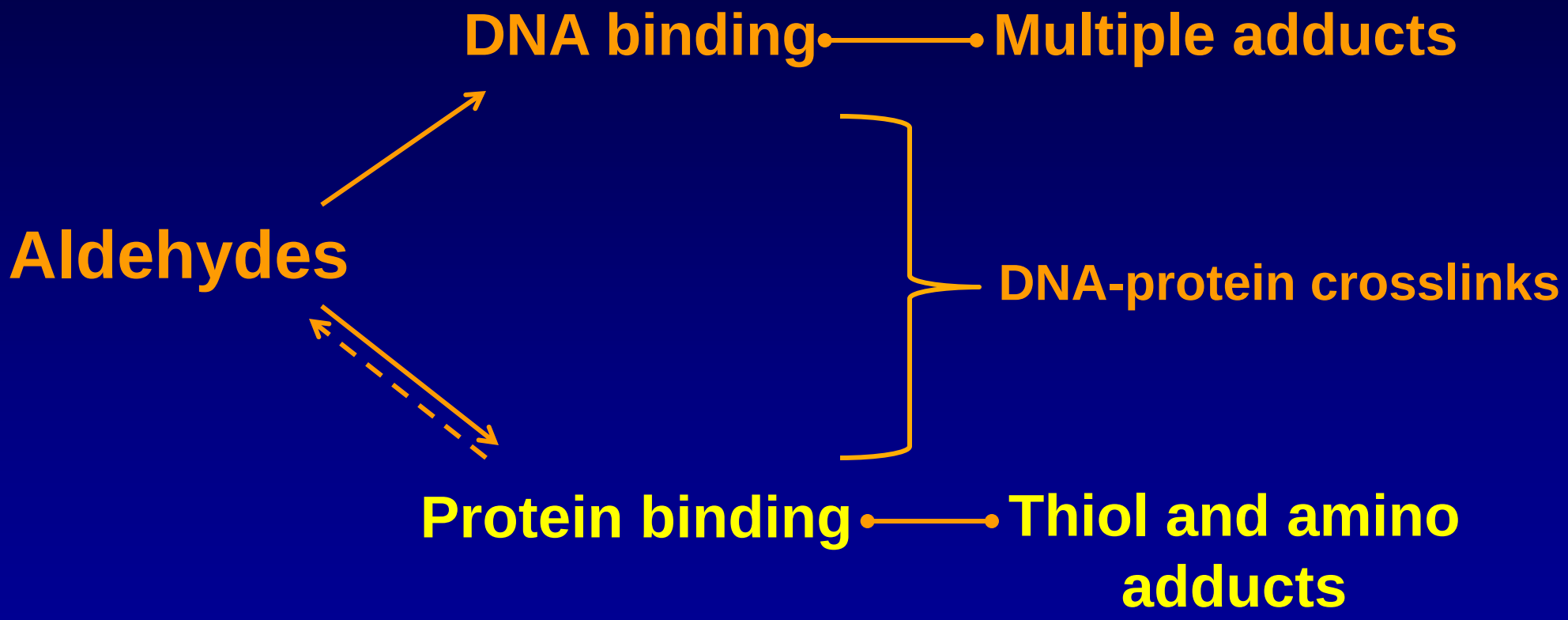
NTP, 2008

Examples of DNA Adducts to Guanine and the Phosphate Backbone



Singh and Farmer (2006) Carcinogenesis 27:178-196

Common Reactions of Aldehydes



Mouse Lung Carcinogens

Epoxides or Bioactivated to Epoxides

- Ethylene oxide
- Glycidol
- Acrylamide
- Butadiene
- Chloroprene
- Urethane
- Vinyl chloride

Bioactivation Involving Quinones and/or Epoxides

- Benzene
- Benzofuran
- Cumene
- Ethyl benzene
- Naphthalene
- Styrene

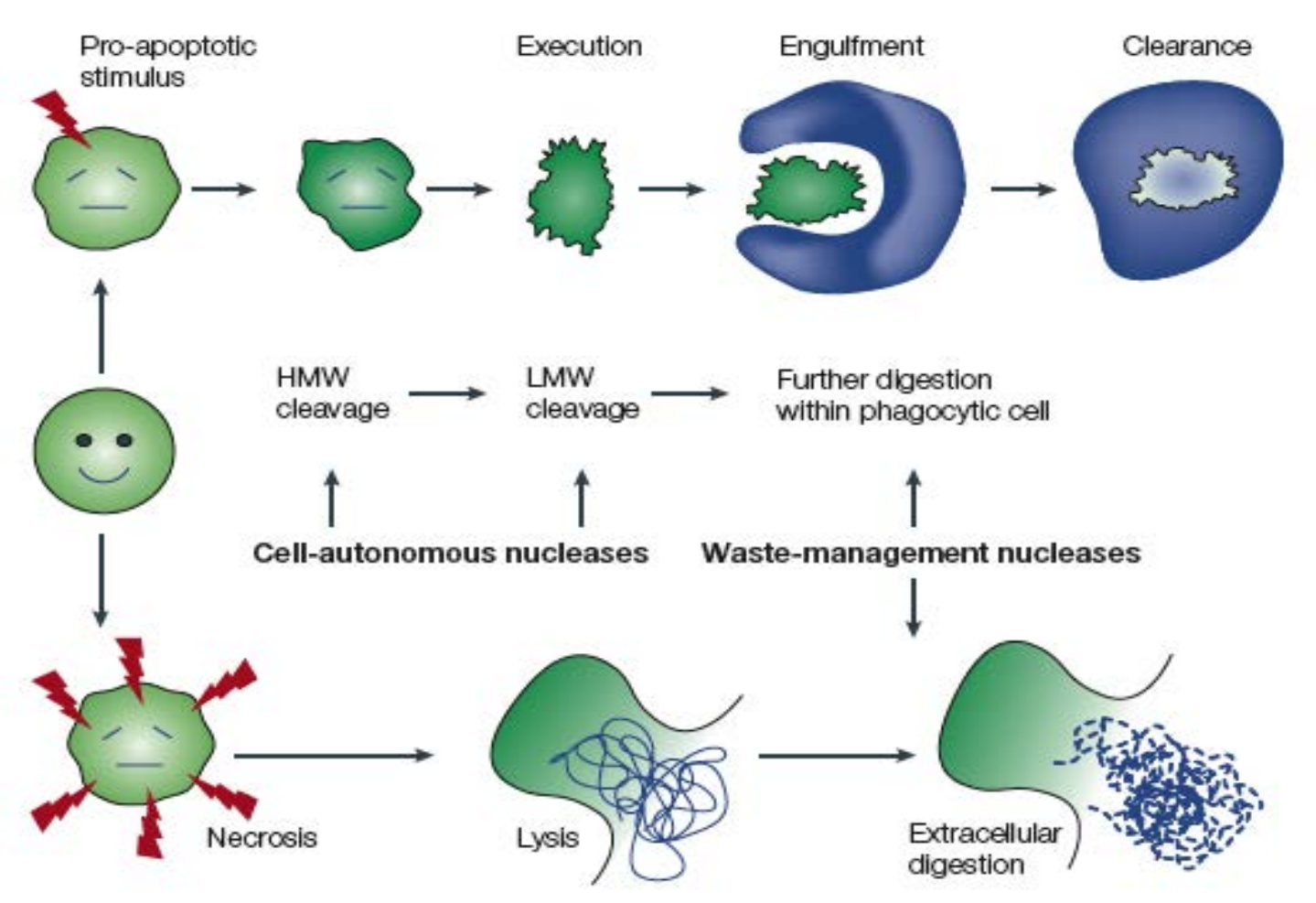
Cytotoxicity and Genotoxicity

- There are multiple reports of DNA strand breakage and fragmentation induced by naphthalene. Similar results can be seen for other agents and endpoints.
- It is important to to consider the occurrence and impact of cytotoxicity in the interpretation of genotoxicity test data for risk assessment.
- The frequency of positive results considered to be irrelevant for predicting cancer risks is believed to increase significantly when high levels of cytotoxicity are seen. Maximum acceptable levels of toxicity have been established for the most common assays.

Cytotoxicity (cont.)

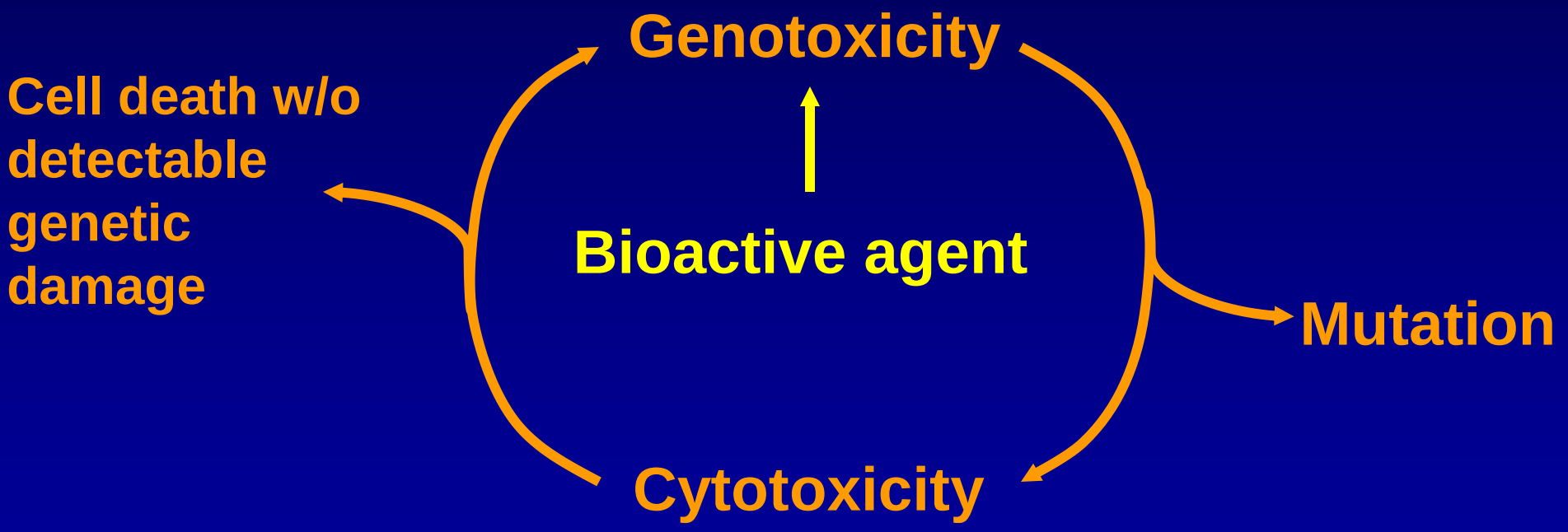
- Adherence to cytotoxicity guidelines which were largely developed for screening chemicals, may be less useful in evaluating the risks of identified carcinogens. For example at a given dose, cytotoxicity frequently occurs at the site where a tumor may later develop. In this case, it would seem unwise to discount genotoxic effects that are also observed at that target site.
- The relevance of genotoxicity results, as influenced by cytotoxicity, exists along a continuum, and using a single cut-off point would seem to be overly simplistic.

Apoptosis, Necrosis and DNA Breakage



Samejima and Earnshaw (2005) Nature Reviews Molecular Cell Biology 6: 677-688

Interrelationship between Cytotoxicity and Genotoxicity



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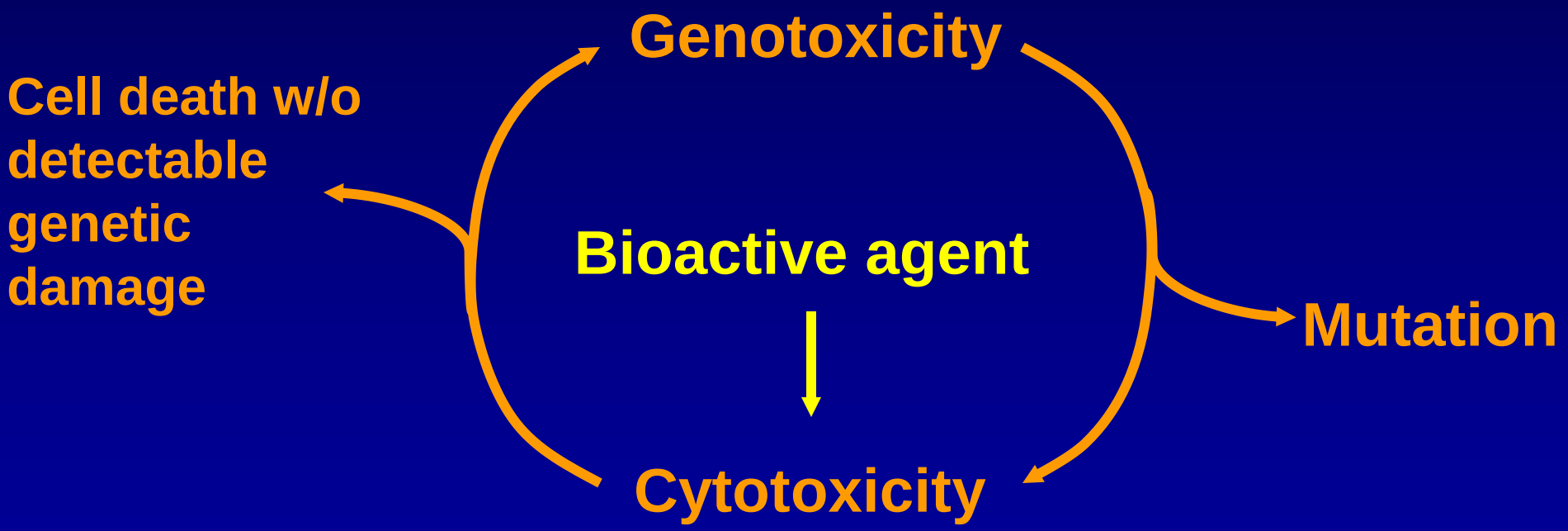

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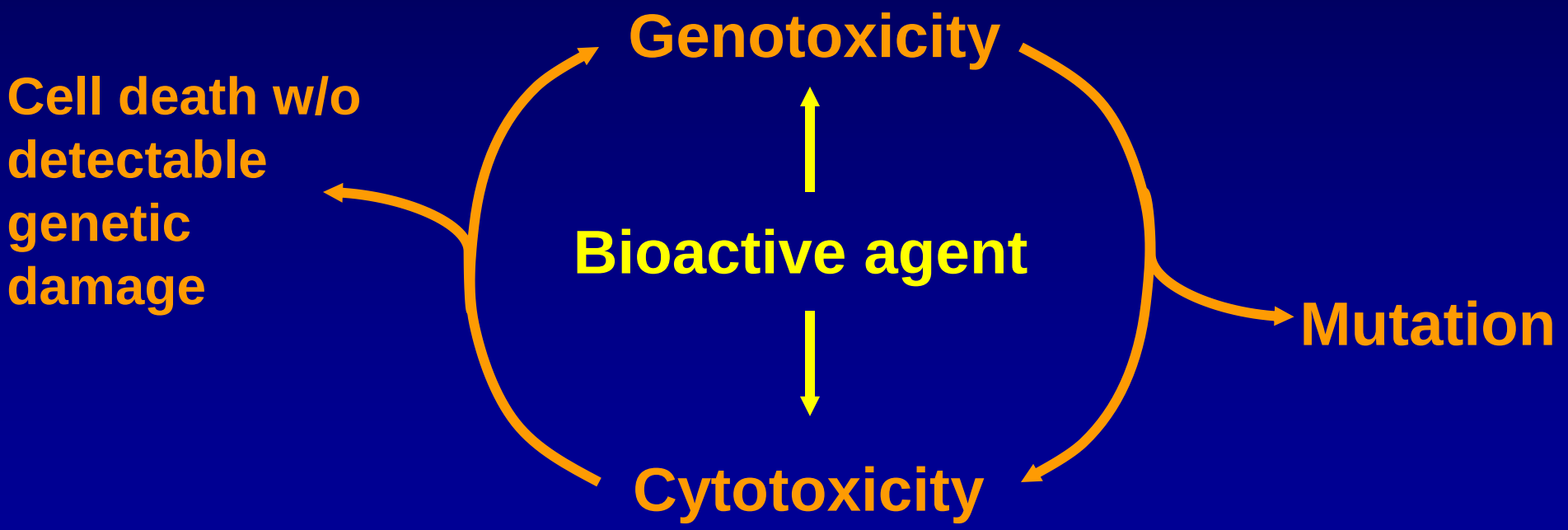
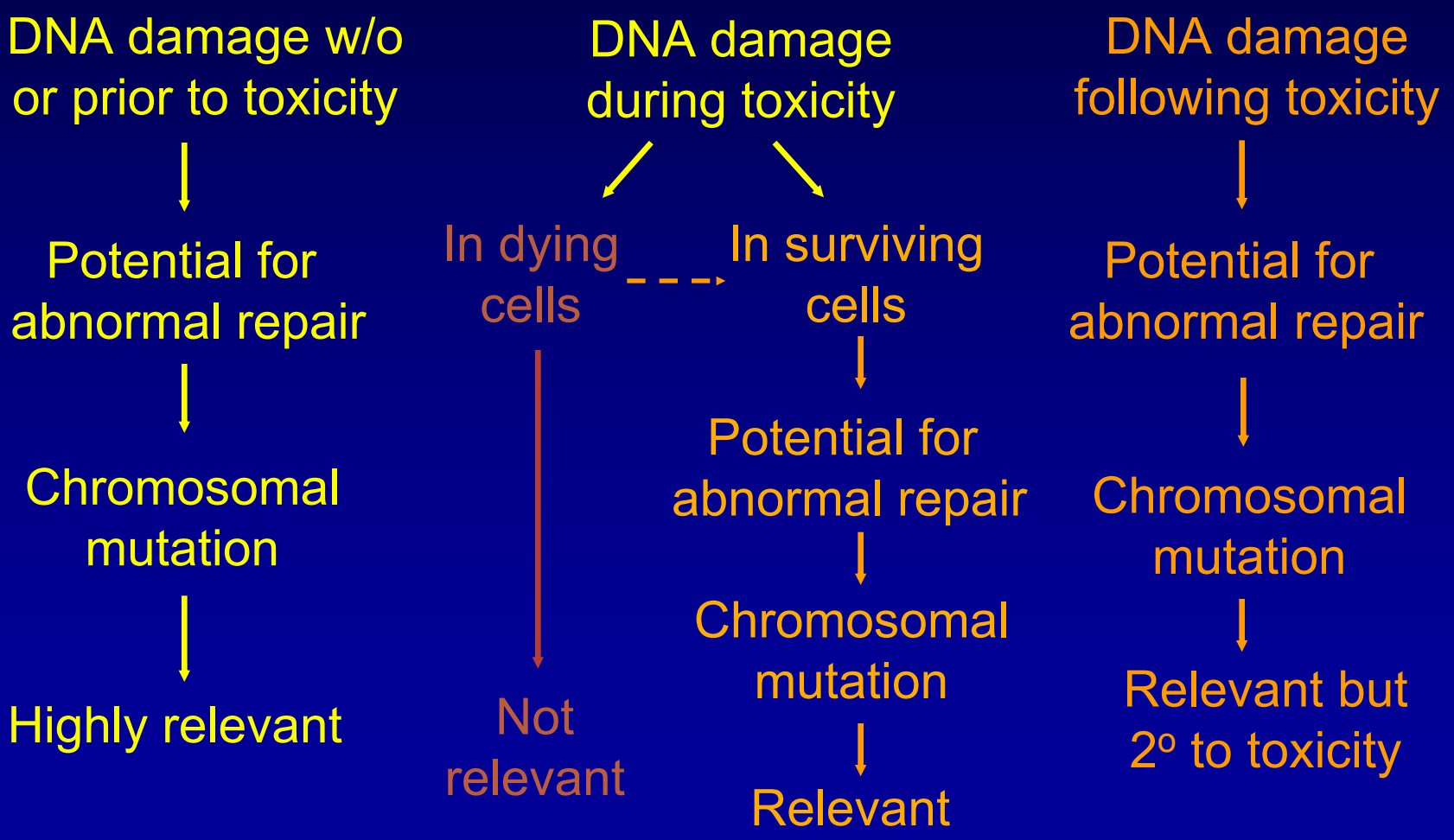
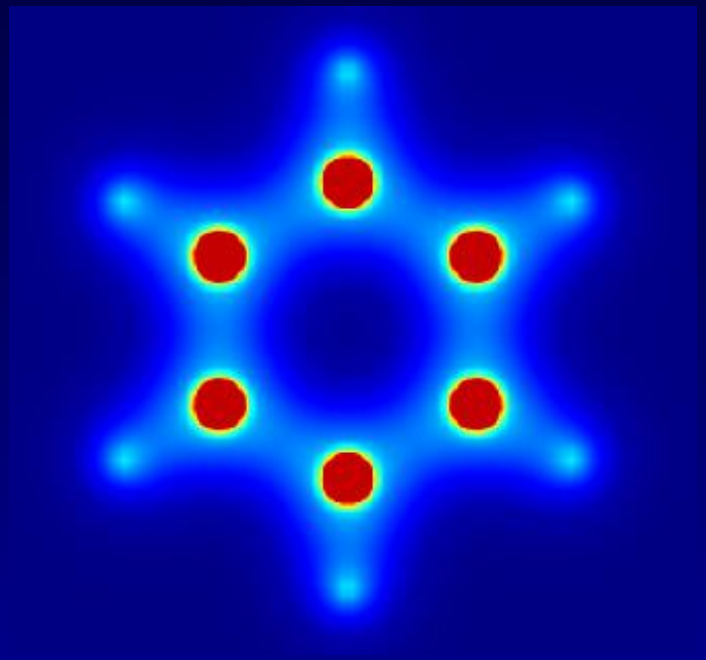


Relevance of Chromosomal Damage





Thank you for your attention.

Questions?