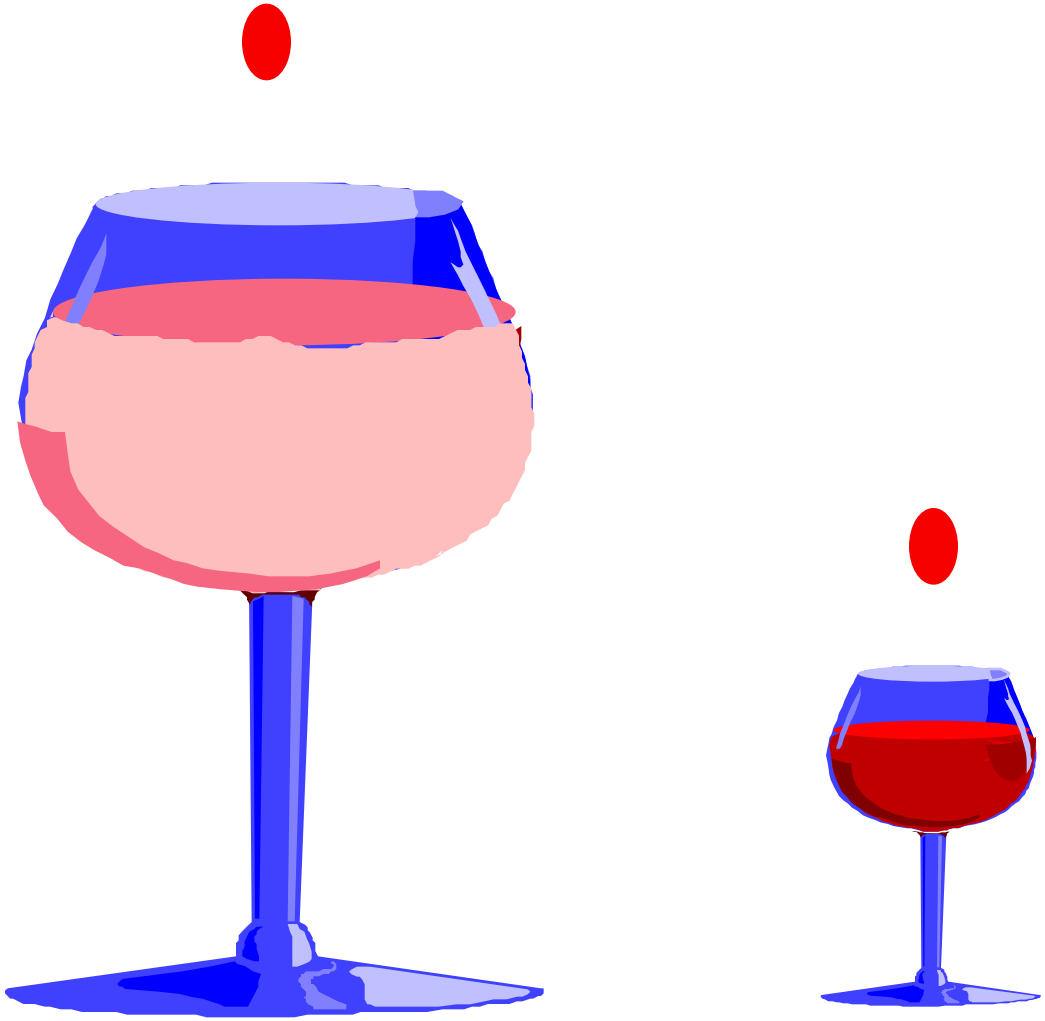
Dose Responses

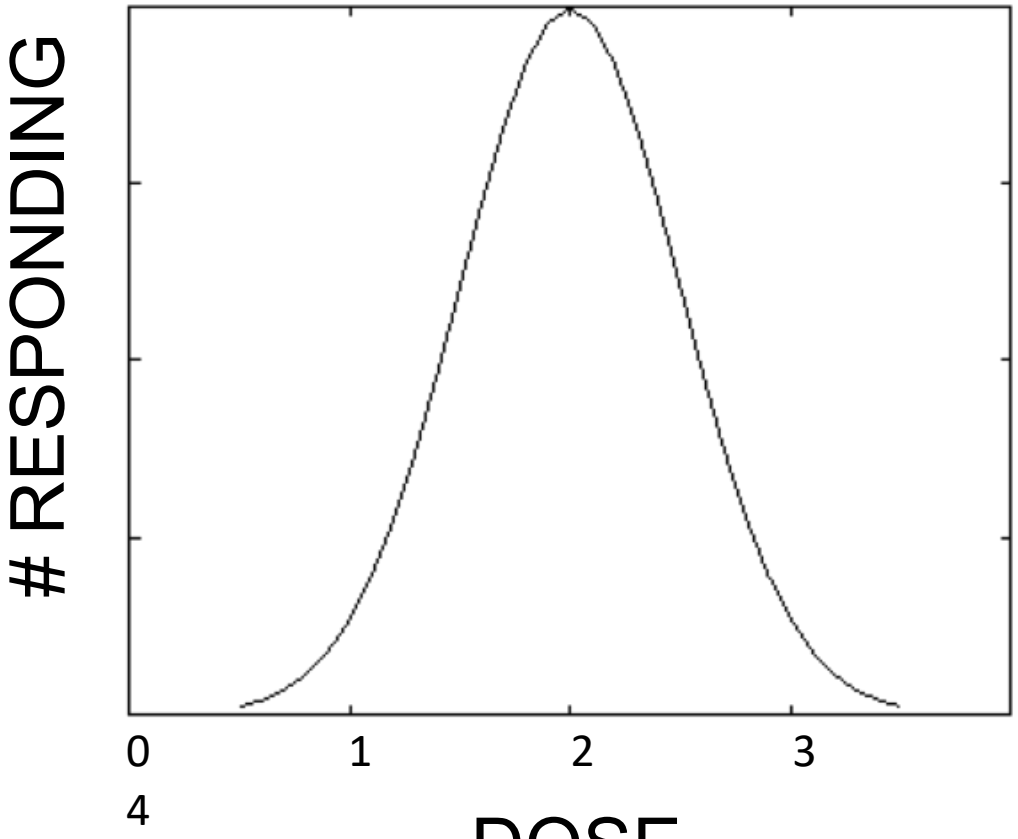
Mechanisms and Targets

Effects of Amount (Dose) on Response

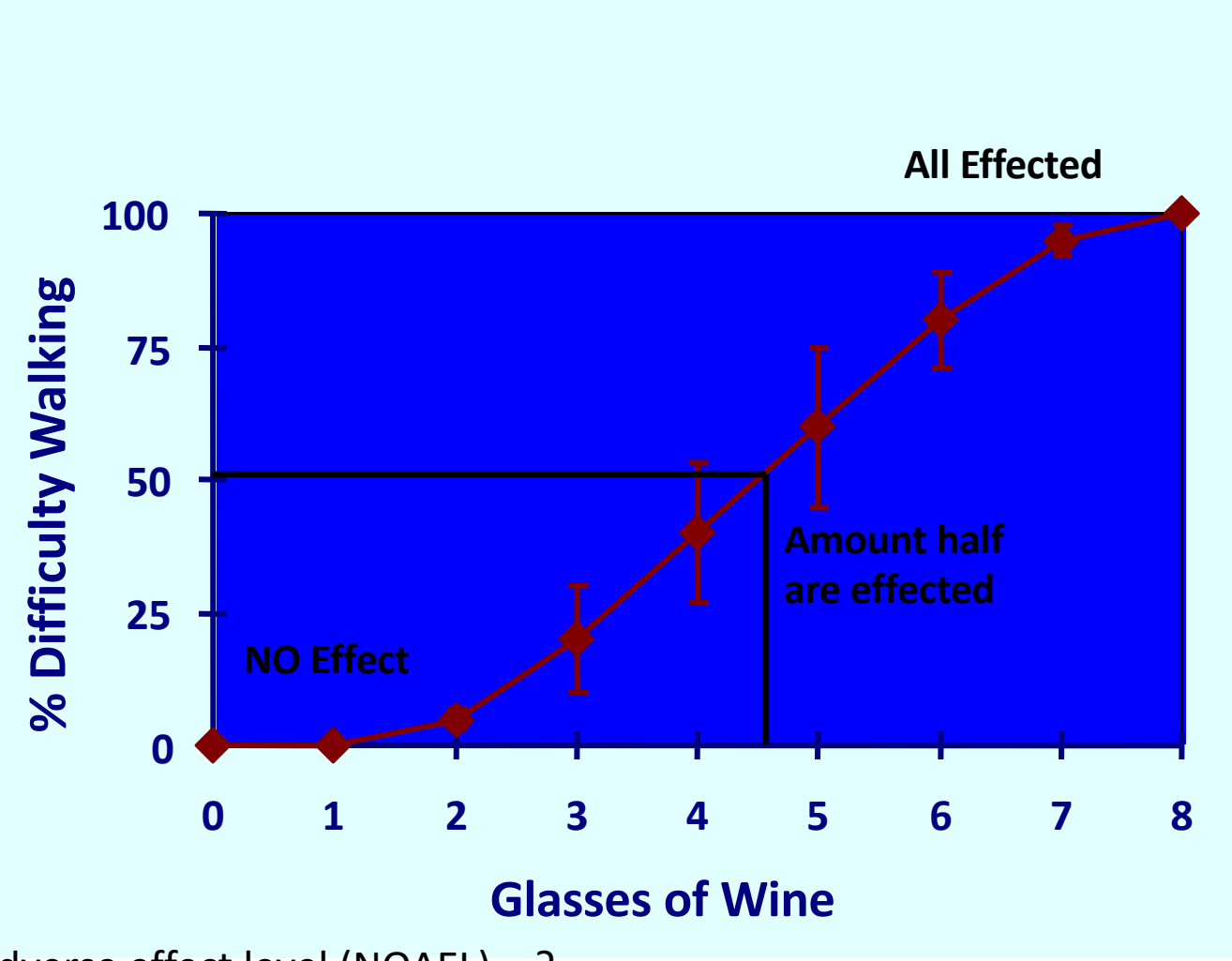
Effects of Size on Response



Differences in a Toxic Response Across a Population



Glasses of Wine - Dose Response
Ex: classical "sigmoid" (S) dose response curve



No adverse effect level (NOAEL) = ?

Lowest adverse effect level (LOAEL) =?

TD_{50} (toxic dose that affects 50% of population =?

Dose-Response Terms

LD_{50} – lethal dose that causes 50% death in a group or population

ED_{50} – effective dose that causes 50% to have desired response (beneficial drug)

EC_{50} – effective concentration that causes 50% response (beneficial or adverse)

TD_{50} – toxic dose that causes 50% to have adverse response (more appropriate than using EC_{50} for a toxic response.

NOAEL – no observed adverse effect level of a chemical

LOAEL – low observed adverse effect level of a chemical

Benchmark dose – usually the 10% response level, extrapolated from a dose-response curve

Assumptions for dose responses

- The agent in question really causes the response
- The magnitude of response is dose related
- The response is quantifiable and can be accurately measured

Reading the Dose-Response Curve Tea Leaves

- Look at the axes
- Scale and shape of graph
- Units (mg vs. ug?)
- Look at shape of line or curve

Same Data, Different Plots

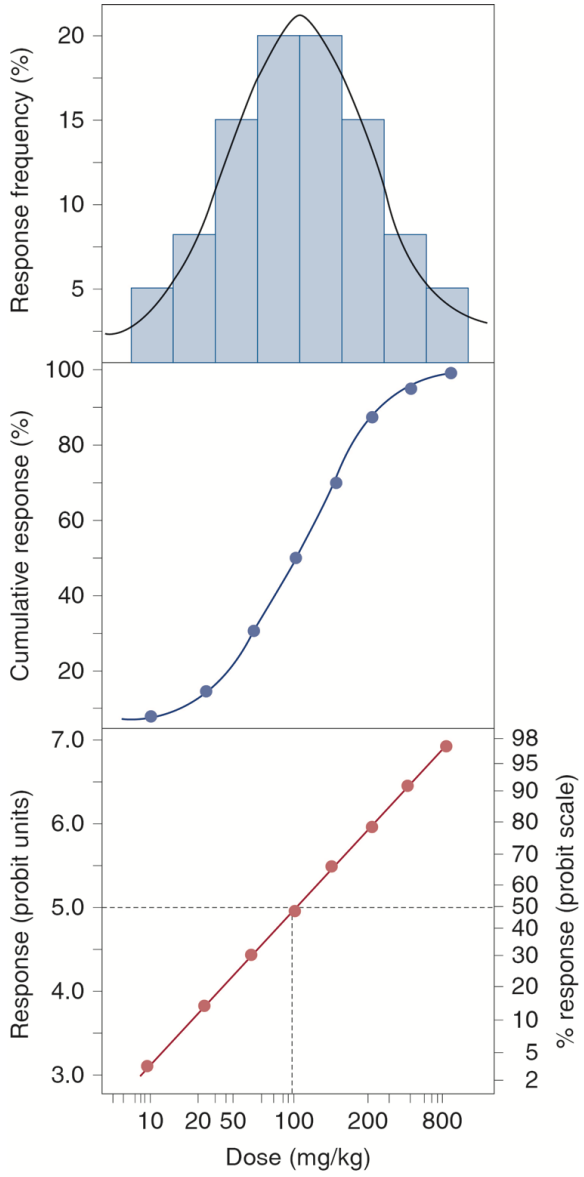


Figure 2-4. *Diagram of quantal dose–response relationship.* The abscissa is a log dosage of the chemical. In the top panel the ordinate is response frequency, in the middle panel the ordinate is percent response, and in the bottom panel the response is in probit units (see text).

Potency & Efficacy

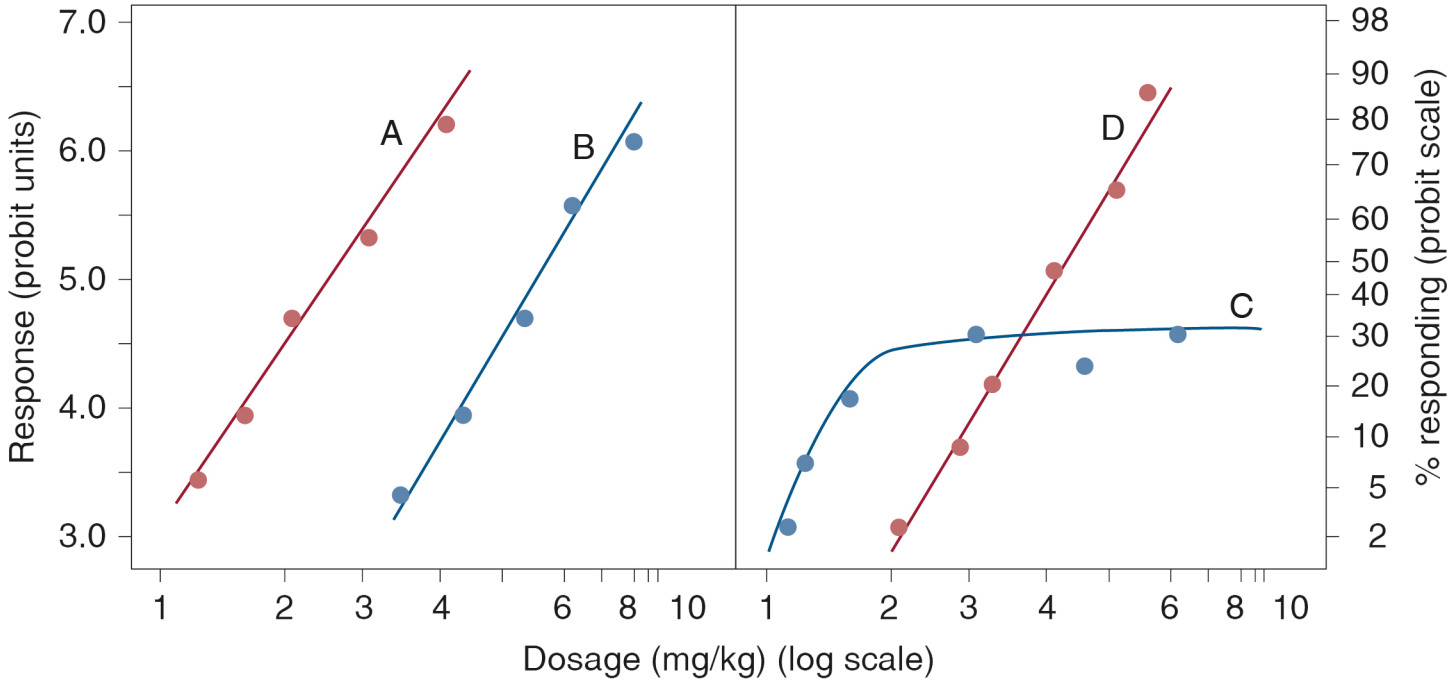


Figure 2-12. *Schematic representation of the difference in the dose–response curves for 4 chemicals (**A-D**), illustrating the difference between potency and efficacy (see text).*

Irregular, non-monotonic, hormetic curves

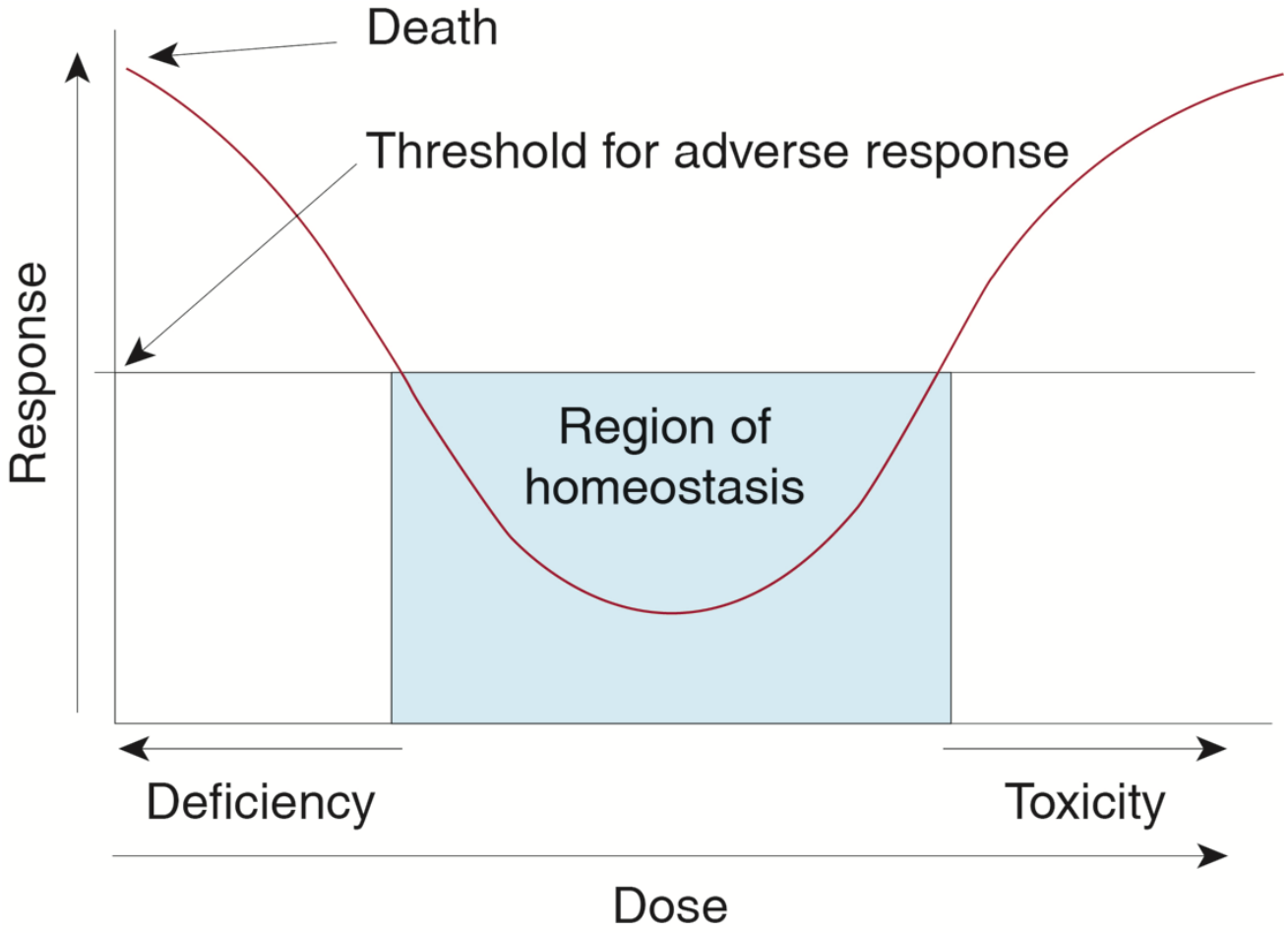


Figure 2-6. *Individual dose–response relationship for an essential substance such as a vitamin or trace element.* It is generally recognized that, for most types of toxic responses, a threshold exists such that at doses below the threshold, no toxicity is evident. For essential substances, doses below the minimum daily requirement, as well as those above the threshold for safety, may be associated with toxic effects. The blue shaded region represents the "region of homeostasis"—the dose range that results in neither deficiency nor toxicity.

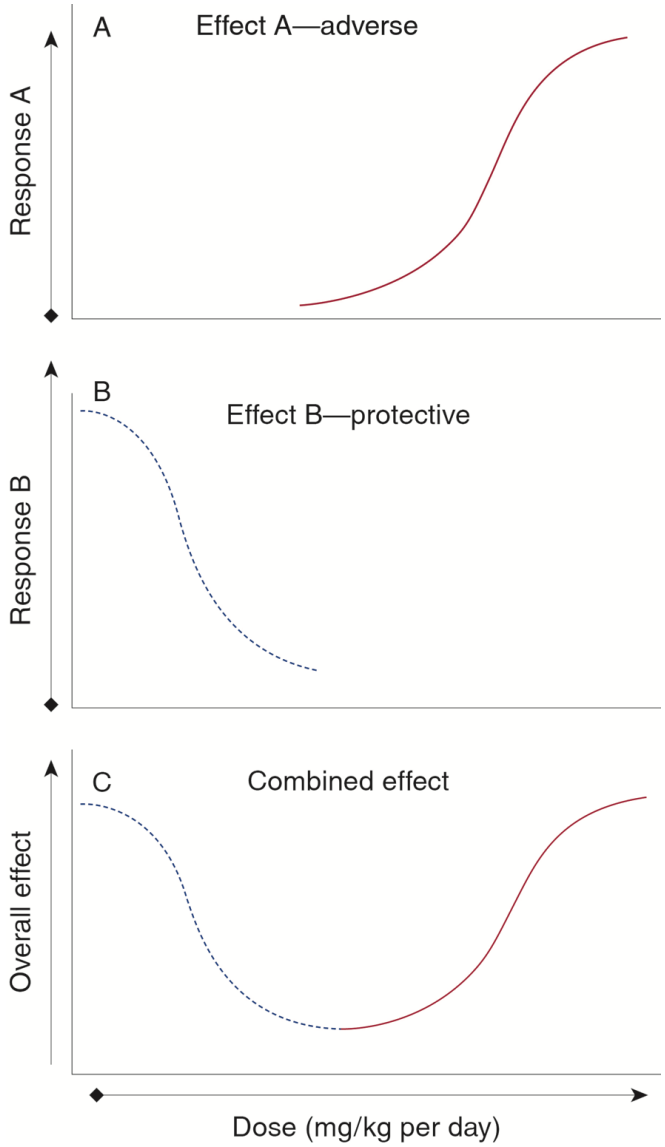


Figure 2-7. *Hypothetical dose–response relationship depicting characteristics of hormesis.* Hormetic effects of a substance are hypothesized to occur when relatively low doses result in the stimulation of a beneficial or protective response (**B**), such as induction of enzymatic pathways that protect against oxidative stress. Although low doses provide a potential beneficial effect, a threshold is exceeded as the dose increases and the net effects will be detrimental (**A**), resulting in a typical dose-related increase in toxicity. The complete dose–response curve (**C**) is conceptually similar to the individual dose–response relationship for essential nutrients shown in Fig. 2-6.

Hormetic Dose Response curve

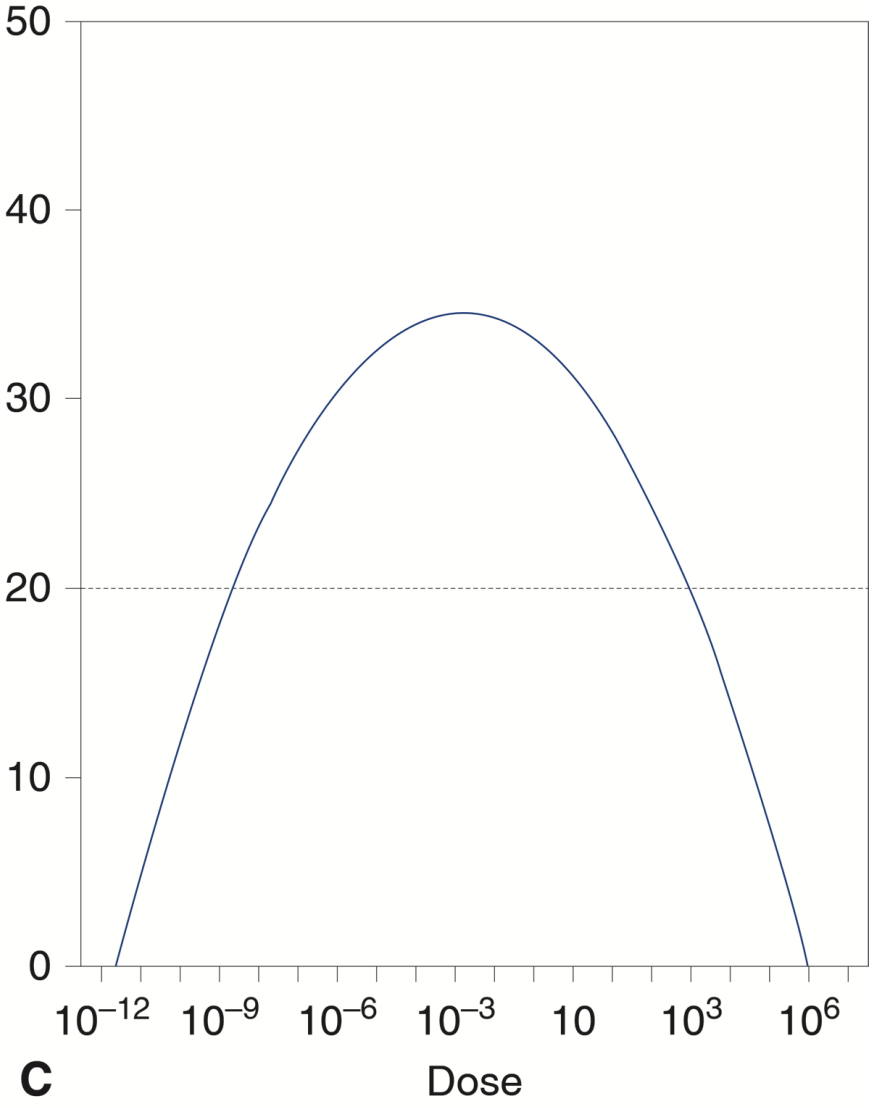


Figure 2-10. *Hypothetical dose–response curves for the (**A**) threshold responses, (**B**) nonthreshold linear response, and (**C and D**) nonmonotonic dose–response (NMDR).* Curves A and B reflect traditional dose–response relationships. However, in the NMDR curve (C), an increase in dose does not necessarily correspond to an increase in response, such that, in this example, doses from 10^{-12} to 10^{-3} dose units result in an increase in response, and doses from 10^{-3} to 10^{6} dose units result in a decrease in response. Curve D represents the NMDR curves observed in mammary gland morphological parameters after administration of estradiol to ovariectomized females. The *left y-axis* is the number of terminal end buds (TEBs), and the *right y-axis* is total area of all TEBs; the TEB is an estrogen-dependent structure. (Based on Vandenberg *et al.*, 2009.)

Estrogen dose response curve

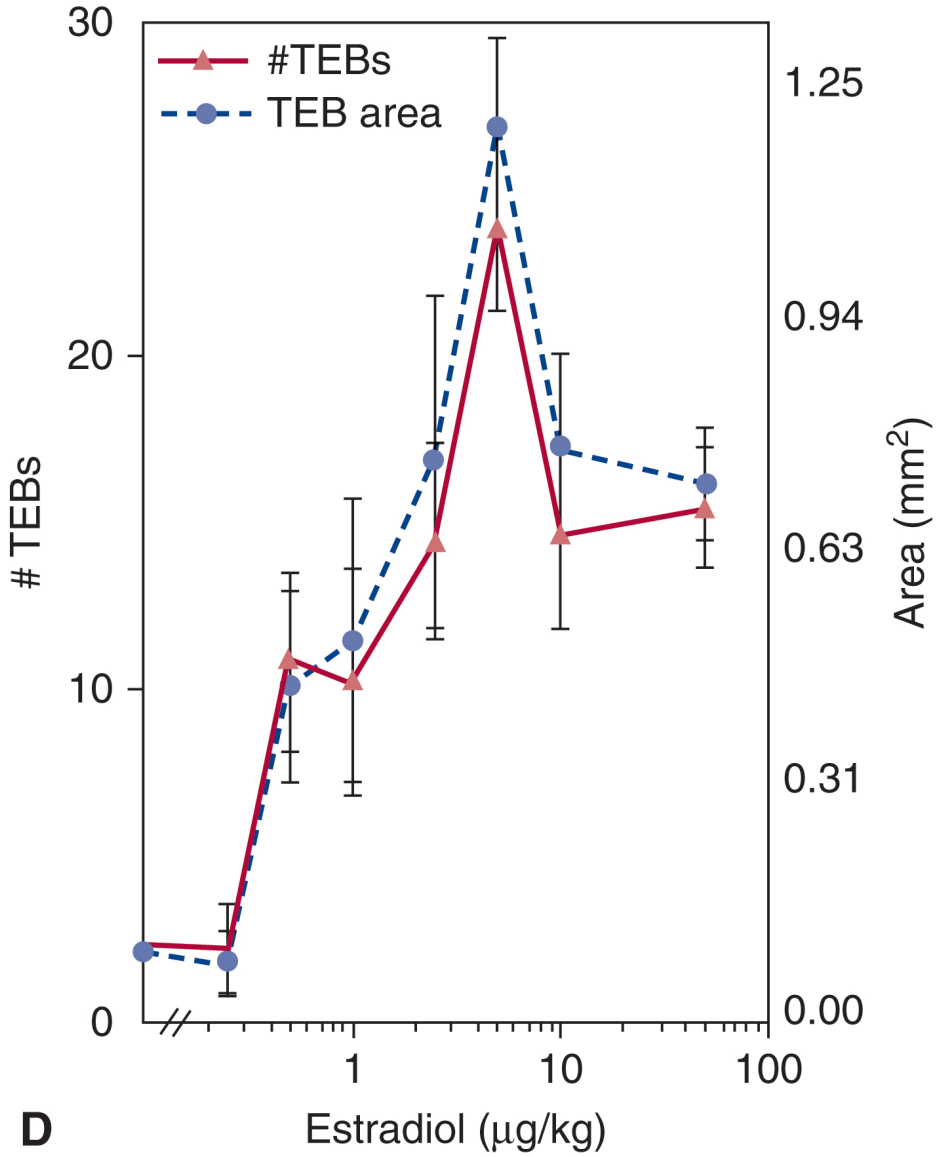
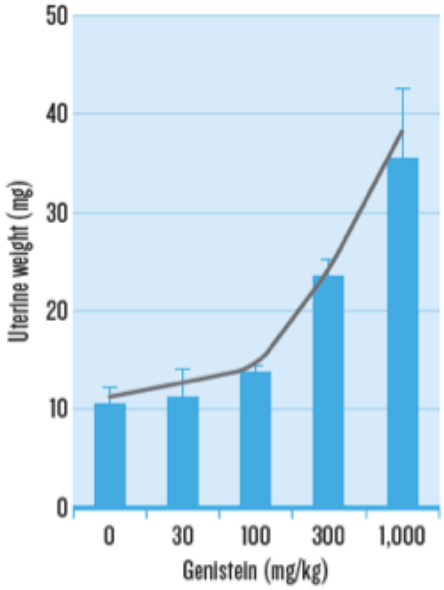
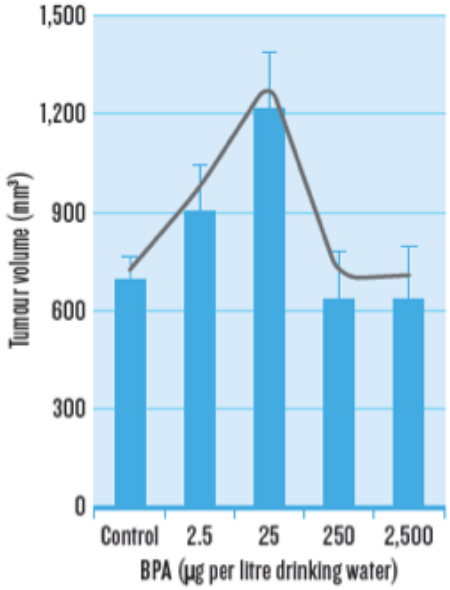


Figure 2-10. *Hypothetical dose–response curves for the (A) threshold responses, (B) nonthreshold linear response, and (C and D) nonmonotonic dose–response (NMDR).* Curves A and B reflect traditional dose–response relationships. However, in the NMDR curve (C), an increase in dose does not necessarily correspond to an increase in response, such that, in this example, doses from 10^{-12} to 10^{-3} dose units result in an increase in response, and doses from 10^{-3} to 10^{6} dose units result in a decrease in response. Curve D represents the NMDR curves observed in mammary gland morphological parameters after administration of estradiol to ovariectomized females. The *left y-axis* is the number of terminal end buds (TEBs), and the *right y-axis* is total area of all TEBs; the TEB is an estrogen-dependent structure. (Based on Vandenberg *et al.*, 2009.)

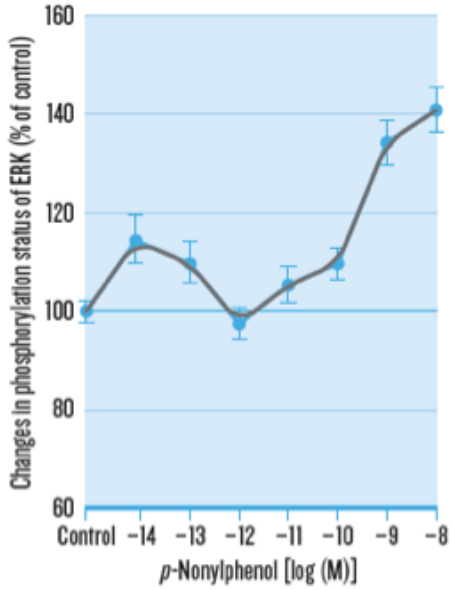
Sometimes dose-responses are complex (non-linear)



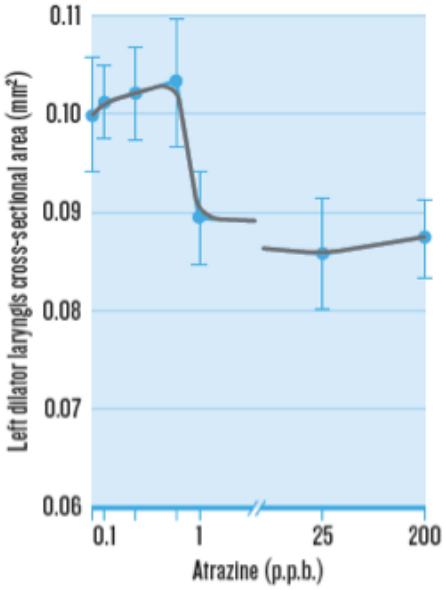
SOURCE: Ohto, R. *et al. J. Toxicol. Sci.* **37**, 879–889 (2012)



SOURCE: Jenkins, S. *et al. Environ. Health Perspect.* **119**, 1604–1609 (2011)



SOURCE: Bulayeva, N. N. & Watson, C. S. *Environ. Health Perspect.* **112**, 1481–1487 (2004)



SOURCE: Hayes, T. A. *et al. Proc. Natl Acad. Sci. USA* **99**, 5476–5480 (2002).

See "The Learning Curve" for review: 2012 NATURE vol. 490, p. 462

And *Review of the Environmental Protection Agency's State-of-the-Science Evaluation of Nonmonotonic Dose-Response Relationships as they Apply to Endocrine Disrupters*, Natl. Acad.Sciences, 2014
http://www.nap.edu/catalog/18608/review-of-the-environmental-protection-agencys-state-of-the-science-evaluation-of-nonmonotonic-dose-response-relationships-as-they-apply-to-endocrine-disrupters

Mechanisms and Targets of Toxicity

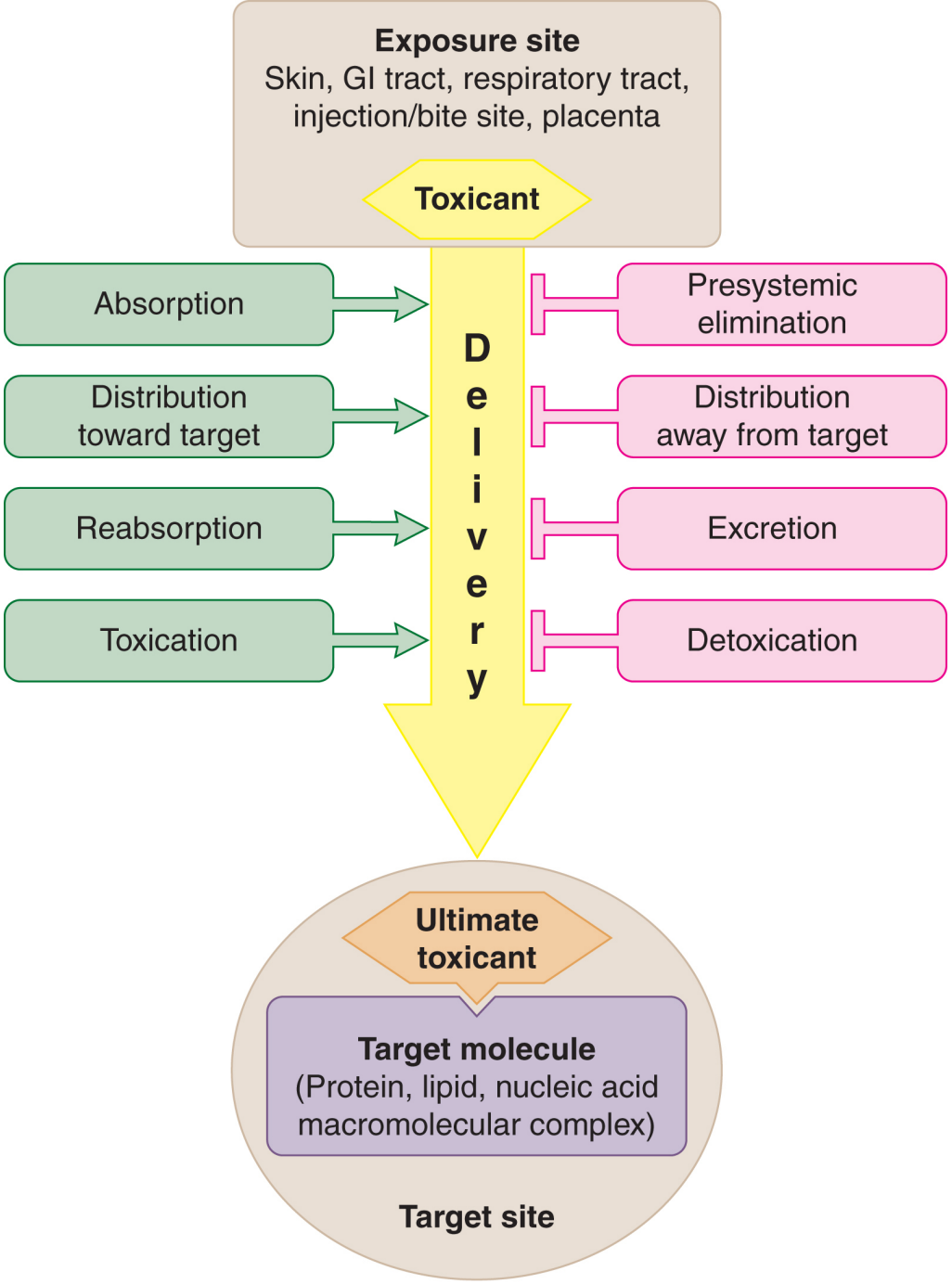


Figure 3-2. *The process of toxicant delivery is the first step in the development of toxicity.*

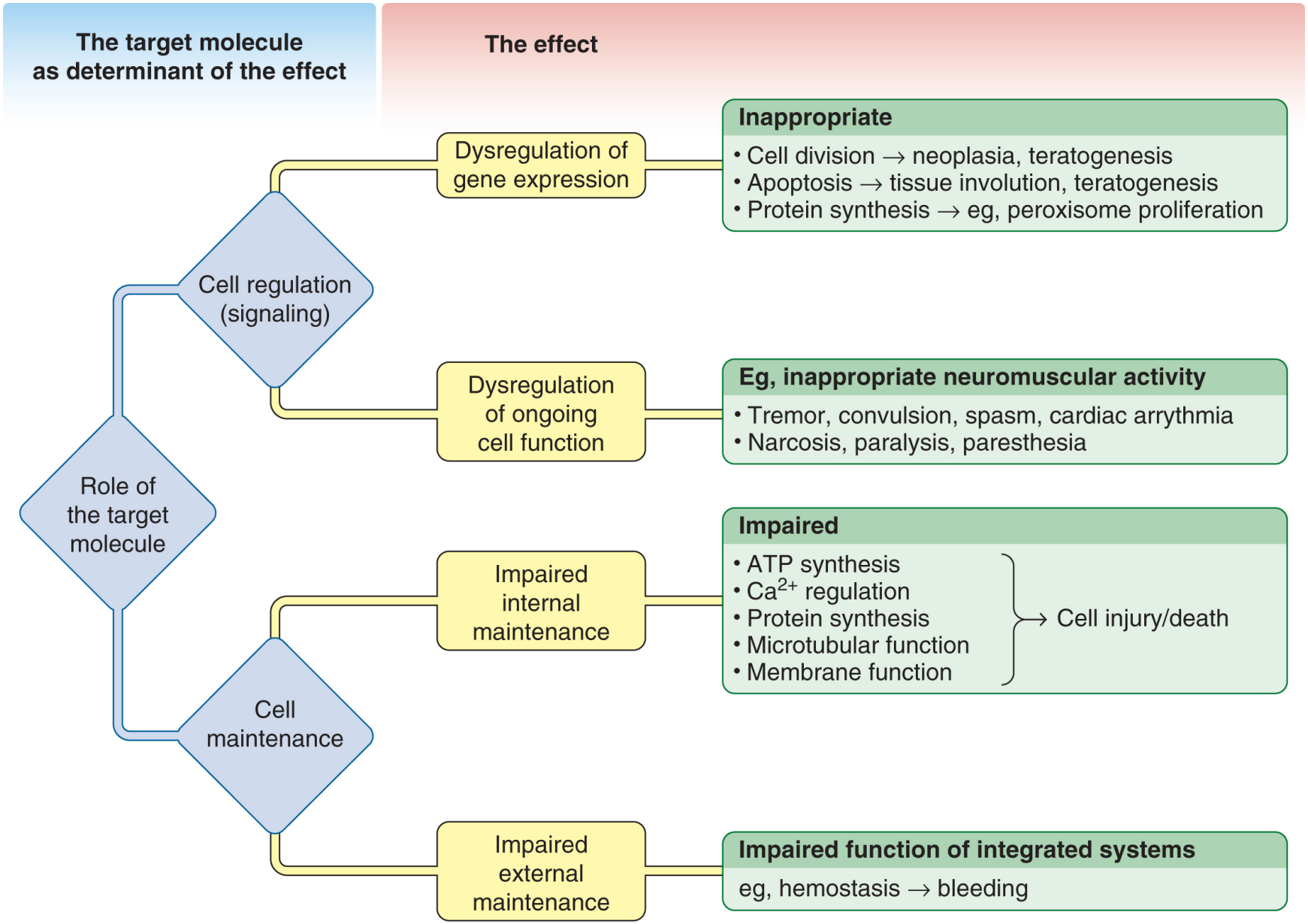


Figure 3-10. *The third step in the development of toxicity: alteration of the regulatory or maintenance function of the cell.*

Oxidation and Reduction of **P**ara**Q**uat (a banned herbicide), **D**oxo**R**ubicin (cancer drug) and **N**itro**F**urantoin (antibiotic)

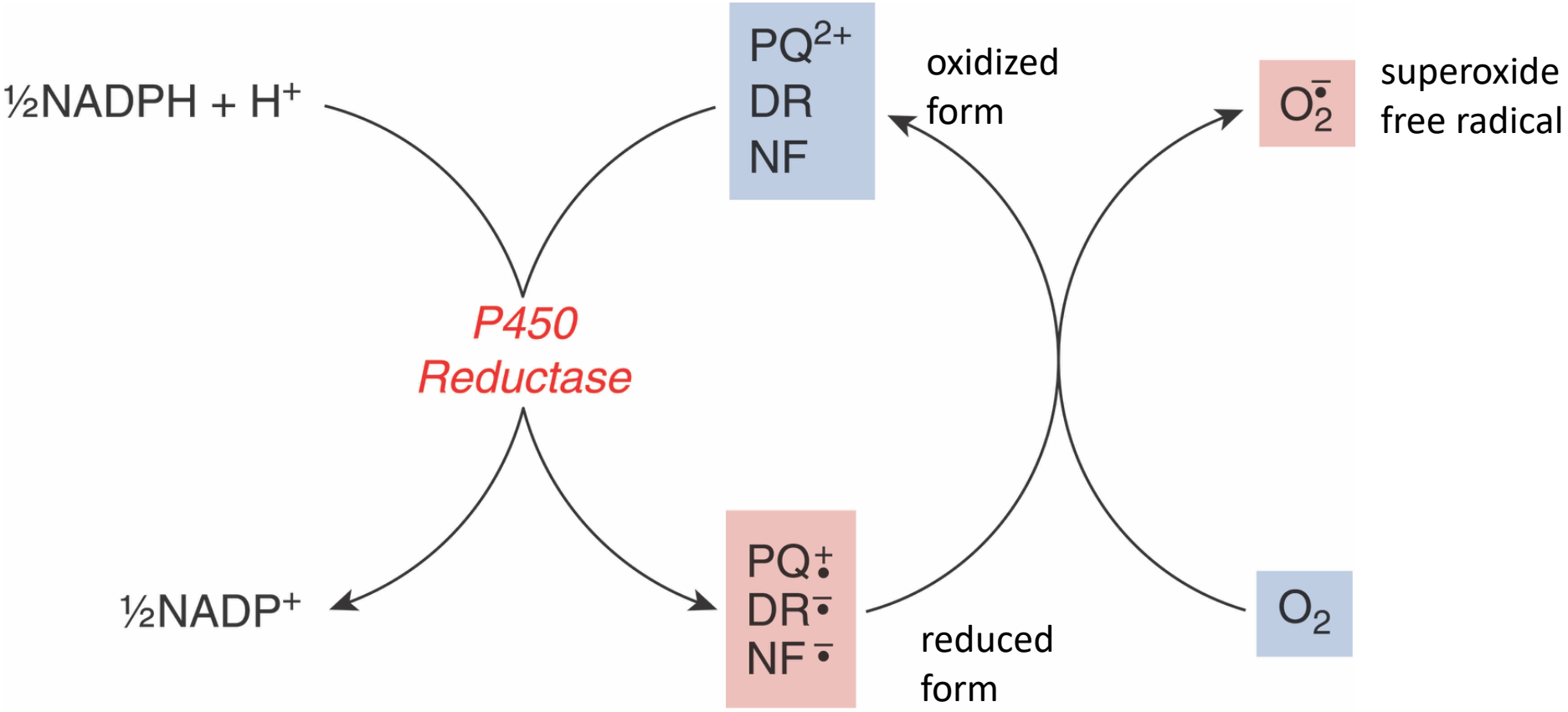
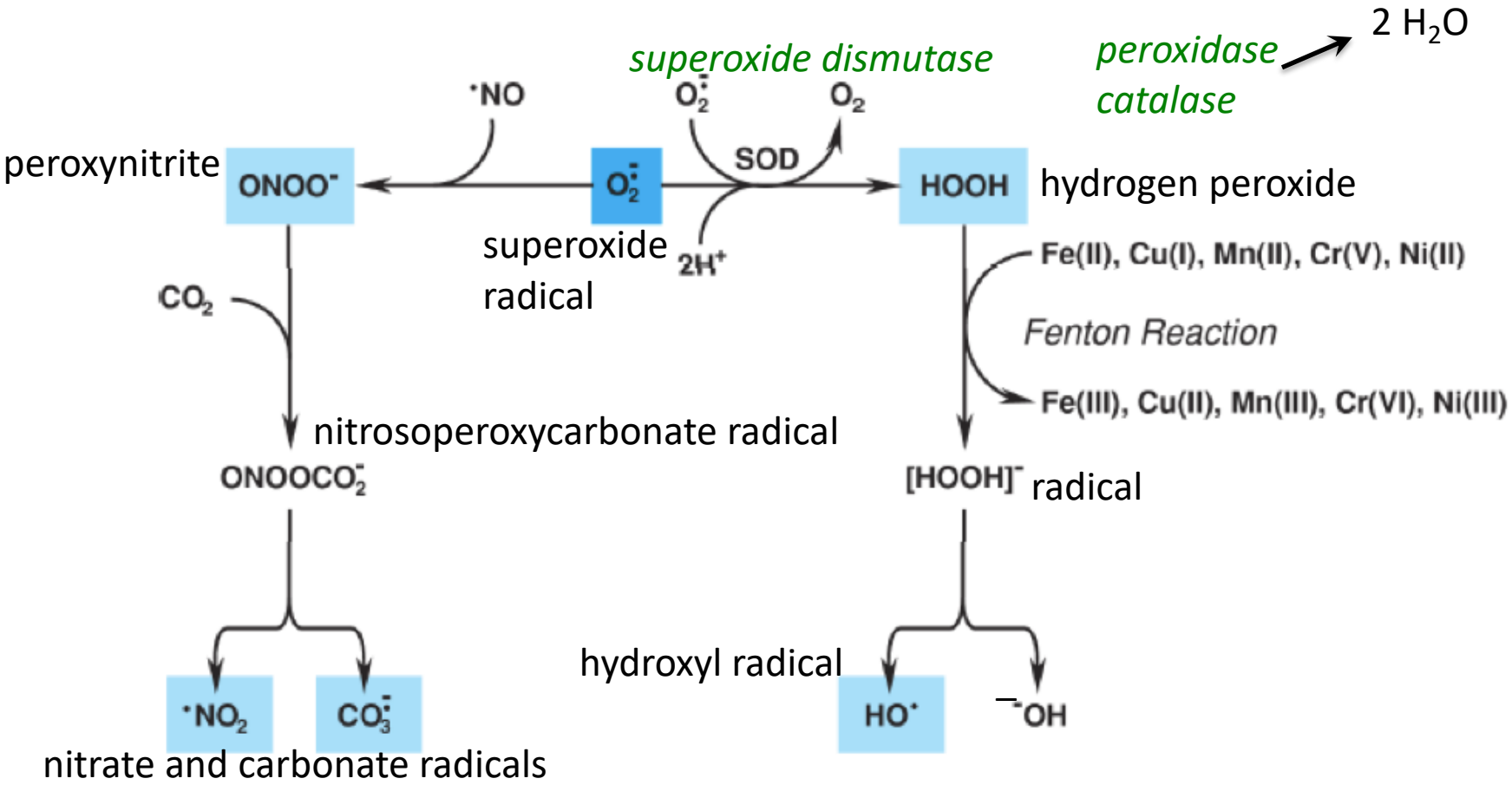


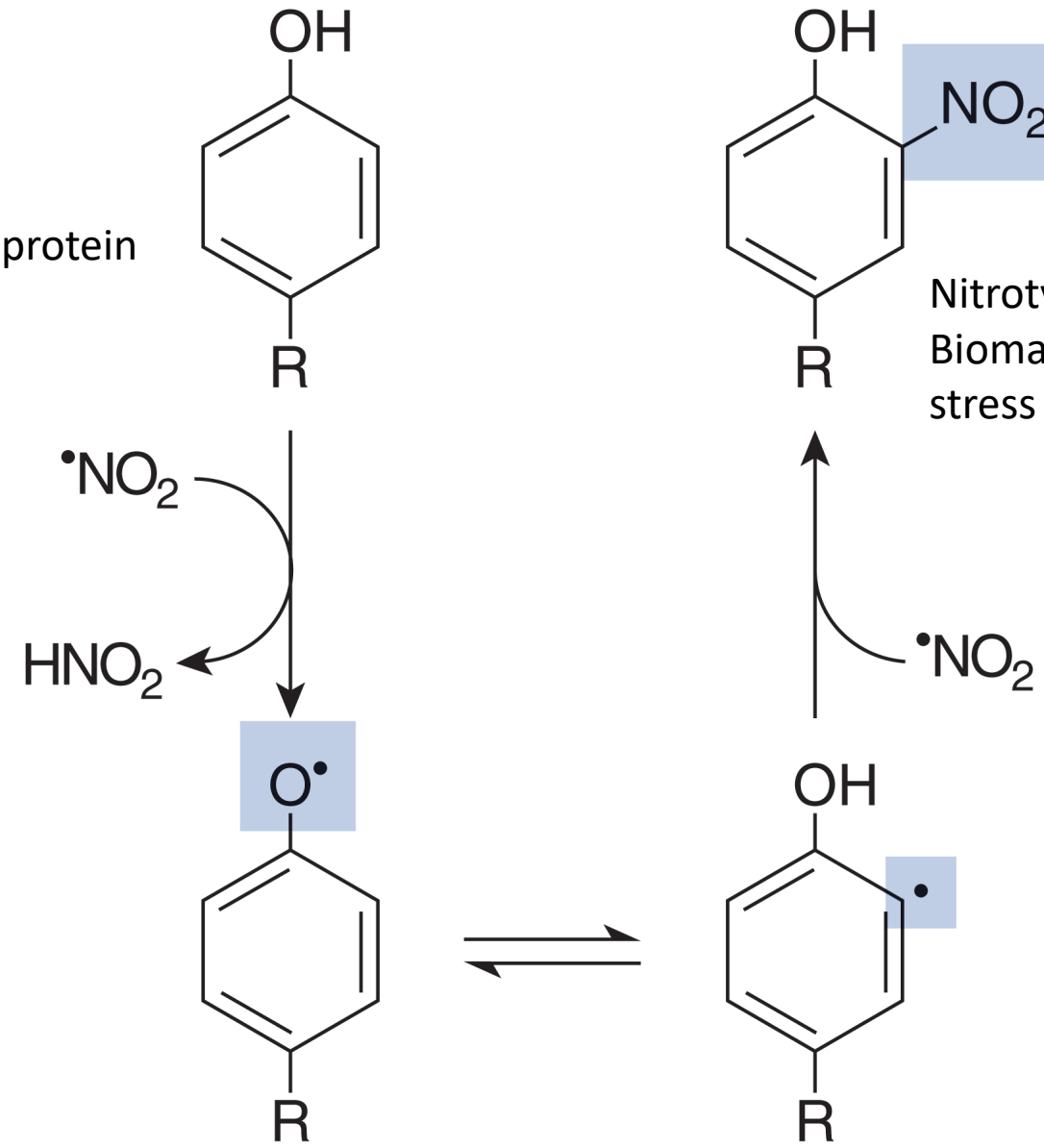
Figure 3-3. *Production of superoxide anion radical ($O_2^{\bullet}$) by paraquat (PQ^{2+}), doxorubicin (DR), and nitrofurantoin (NF).* Note that formation of $O_2^{\bullet}$ is not the final step in the toxication of these xenobiotics, because $O_2^{\bullet}$ can yield the much more reactive hydroxyl radical, as depicted in Fig. 3-4.

Very common mechanism: Oxidative stress and free radical chain rxns.



Radicals react with biological substrates (metabolites, co-factors, amino acids, lipids, carbohydrates, proteins, nucleic acids) and glutathione, metallothionein, ascorbate, tocopherol, retinoids, other antioxidants sequester radicals and are protective.

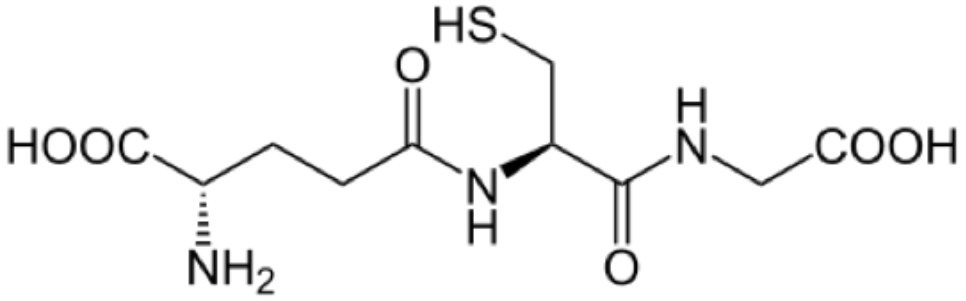
Tyrosine residue in protein



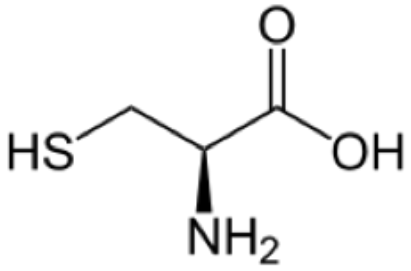
Nitrotyrosine in protein
Biomarker for oxidative stress

Figure 3-8. *Formation of 3-nitrotyrosine residues in proteins by reaction with nitrogen dioxide ($^{\bullet}NO_2$).* $^{\bullet}NO_2$ is an oxidizing and nitrating species generated from $ONOO^-$ (Fig. 3-4). In addition, $^{\bullet}NO_2$ is a contaminant in cigarette smoke, exhaust of gas engines and stoves, as well as the causative agent of "silo-filler's disease."

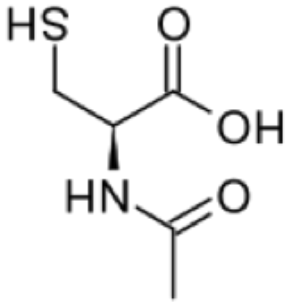
Glutathione, cysteine, and N-acetylcysteine (anti-oxidants) that terminate free radical reactions



Glutathione, a tripeptide



Cysteine, an amino acid

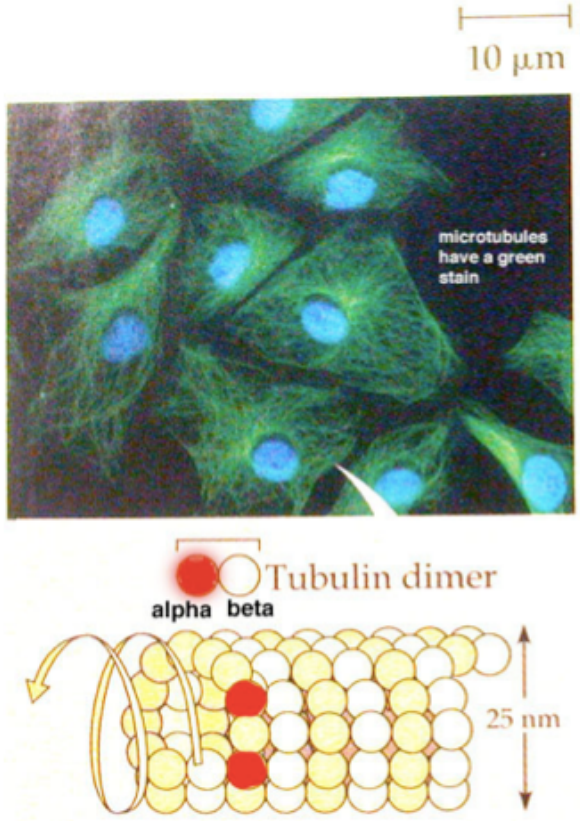


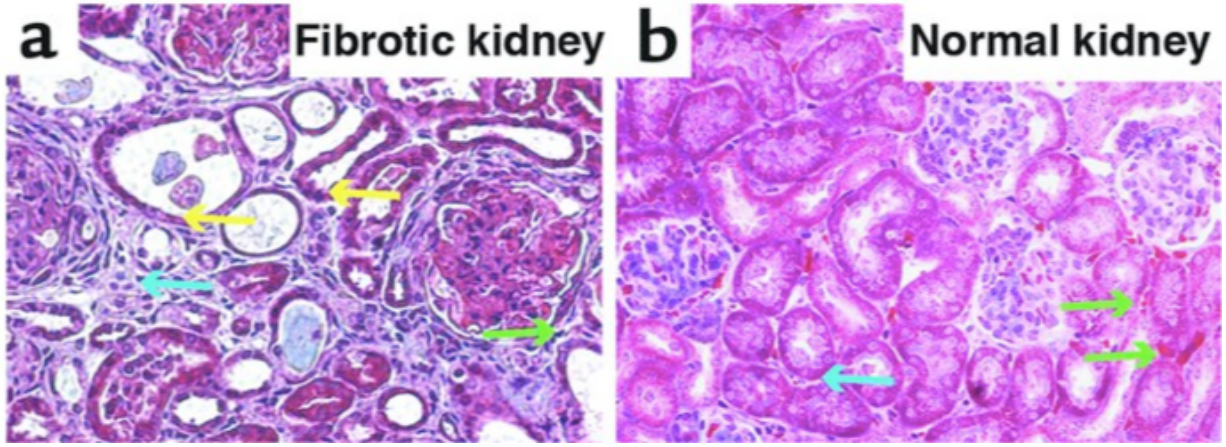
N-acetylcysteine, a drug, antidote

Targets and Mechanisms

- Cellular targets and damage: Mercury ions in nerve cells build up to cytotoxic levels and bind to sulfhydryl groups of key intracellular proteins such as tubulin. Cells die via apoptosis or necrosis.
- Tissue target and damage: Mercury ions damage the renal tubule, causing and subsequent fibrosis.
- Organ targets and damage: Mercury ions cause renal enlargement (hypertrophy), and signs of damage include finding protein, amino acids, and sugars in the urine. In contrast, effects on the brain include cognitive changes and atrophy.

Effects of mercury ions at molecular, cellular and tissue levels





Cellular targets – thousands of them

- Membrane (ion channels, transporters, receptors, lipids, glycoproteins)
- Mitochondria (ion channels, transporters, respiratory chain proteins, DNA, RNA)
- Cytoplasm (enzymes, receptors, ribosomes, structural proteins)
- Nucleus (enzymes, proteins, DNA, RNA)

Changes in gene expression and regulation (major mechanism of toxicity)

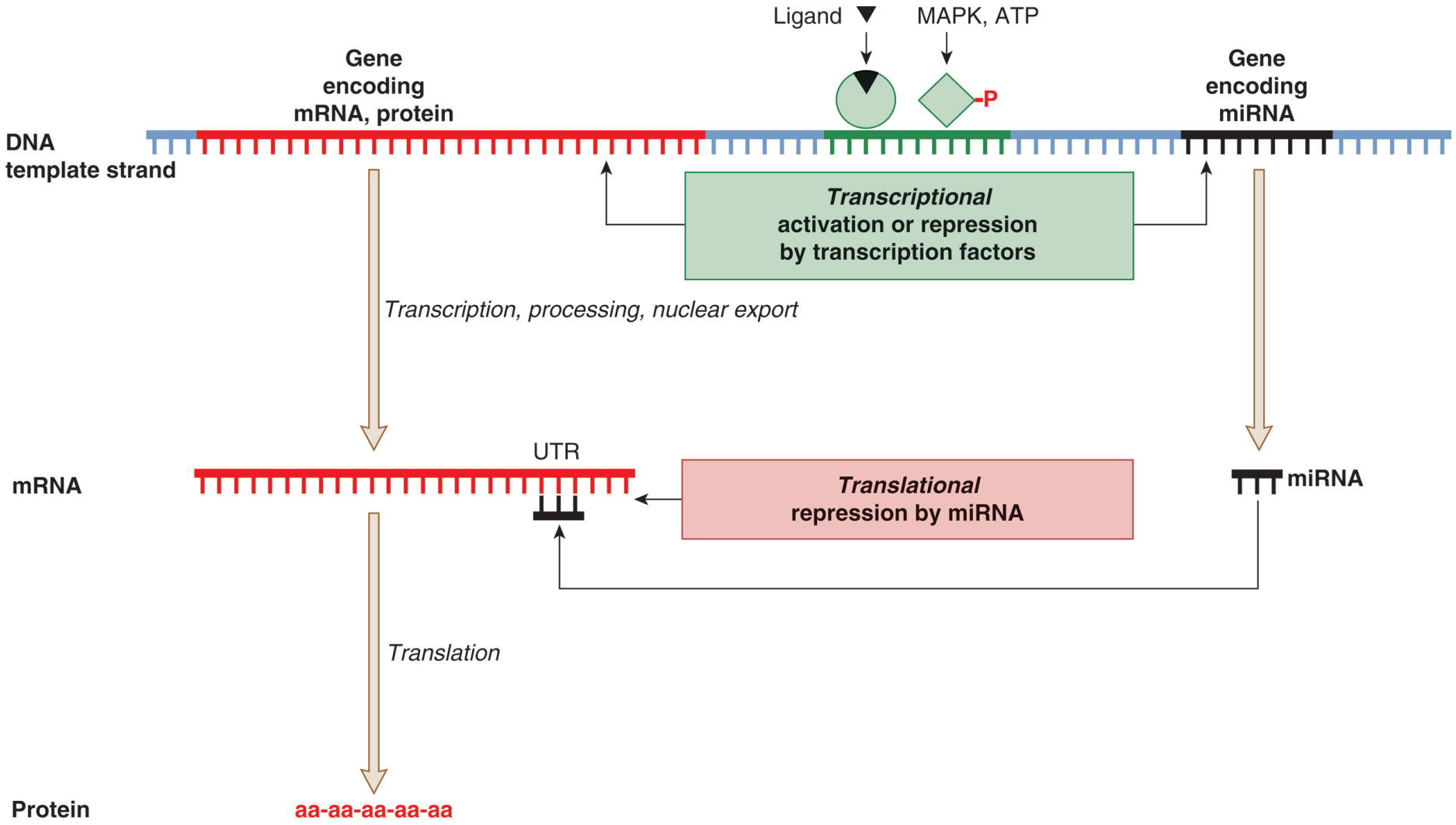


Figure 3-11. *Basic steps of gene expression—transcription factors regulate transcription, whereas microRNAs regulate (repress) translation.* In the process of gene expression, information from a gene is used in the synthesis of a functional gene product. This simplified figure depicts how the synthesis of two important functional gene products, that is, proteins and microRNAs (miRNA, also called small silencing RNA), are interrelated. As described in the text, these normally controlled processes may be dysregulated by endobiotics and xenobiotics as well as during pathologic processes, such as carcinogenesis.

Receptors as targets that contribute to mechanisms of toxicity

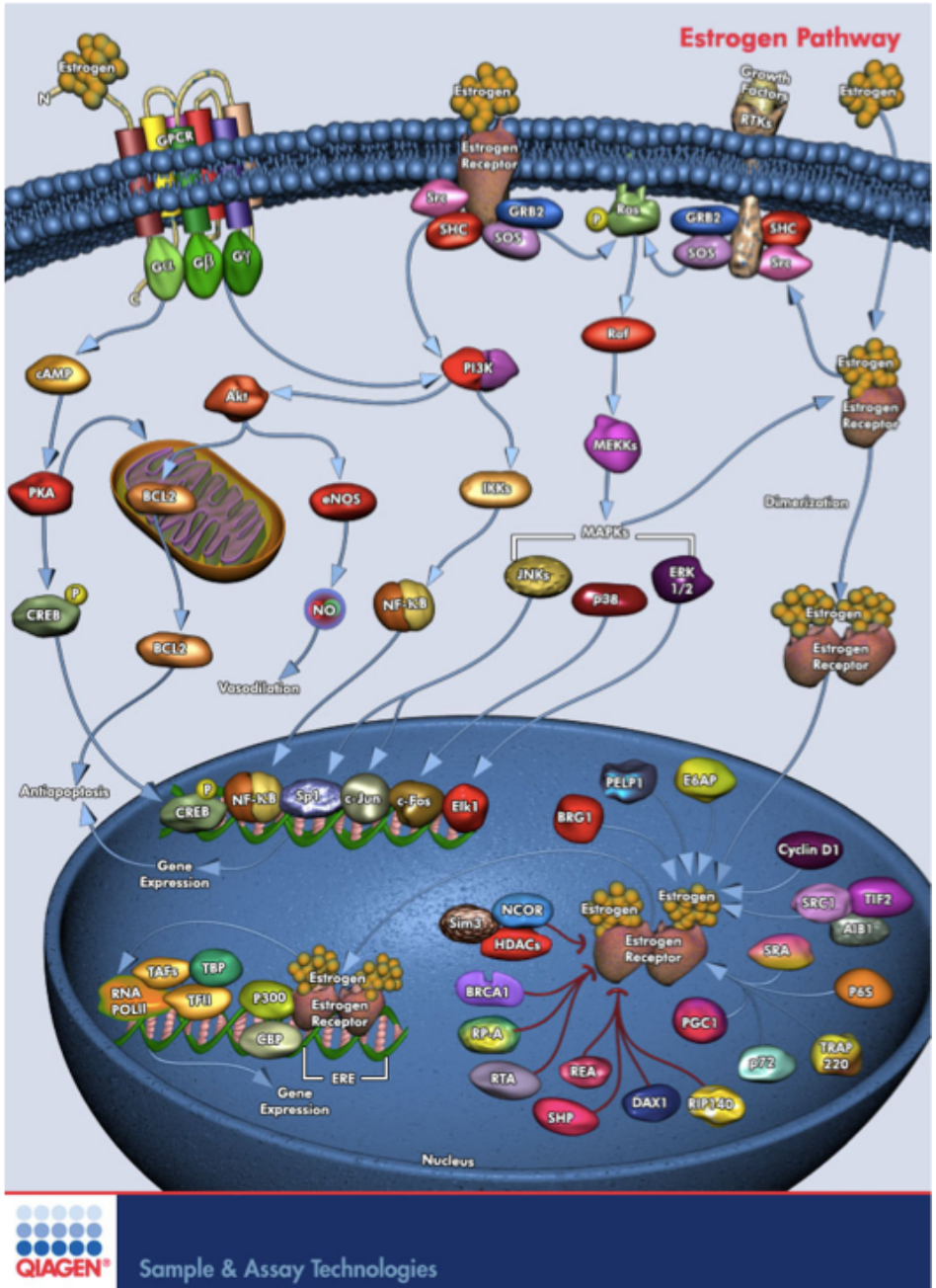
- Receptors (the locks) are usually proteins that act as molecular switches. They control cellular functions by activating or inhibiting various biochemical pathways.
- Ligands (the keys) are endogenous or exogenous molecules (chemicals, often with MW <400) that bind and activate (or block) receptors.
- Some well known receptor-ligand combinations include the acetylcholine ligand-receptor pair (quick acting) and cortisol (ligand)-glucocorticoid receptor pair (slow acting).
- There are thousands of receptors that are present in different cell types throughout the body.

Ligands and Receptors

- Exogenous chemicals, pollutants, etc., can act as ligands, interacting with receptors and switching them on or off at inappropriate times.
- **Ligand concentration must be sufficient to have an effect (Paracelsus: "the dose makes the poison").**
- This interaction leads to inappropriate regulation in cells. Example: "Prepubertal Gynecomastia Linked to Lavender and Tea Tree Oils" (Plant compounds in some lotions interact with estrogen receptors, causing breast development in boys.)

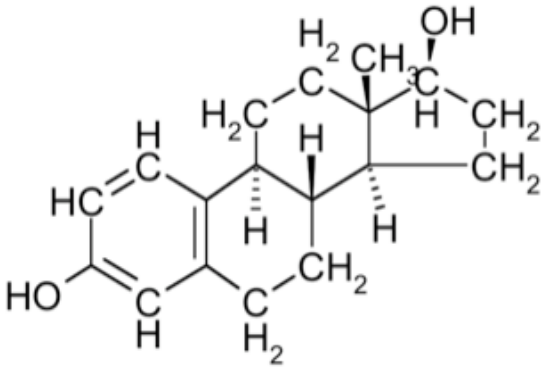
  N. Eng. J. Med 356:479-485, 2007.
- Nicotine (from tobacco) and thousands of other toxic substances can act via receptors to produce toxic effects.

Estrogen Signaling Pathway

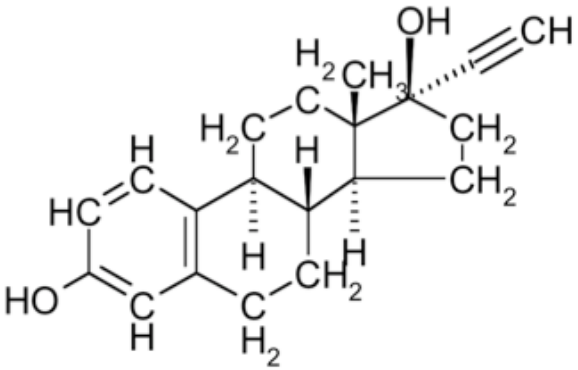


Estradiol and ethynylestradiol

- Natural hormone



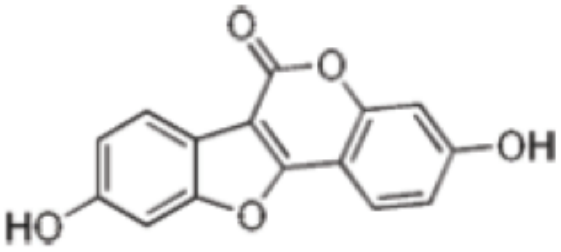
Drug



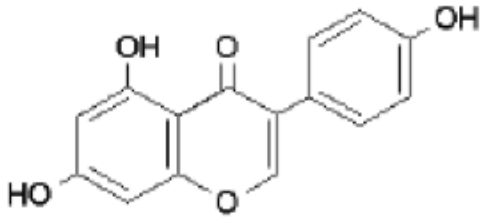
Phyto-estrogens (plants/in diet/“natural”)

Red Clover

Soybean

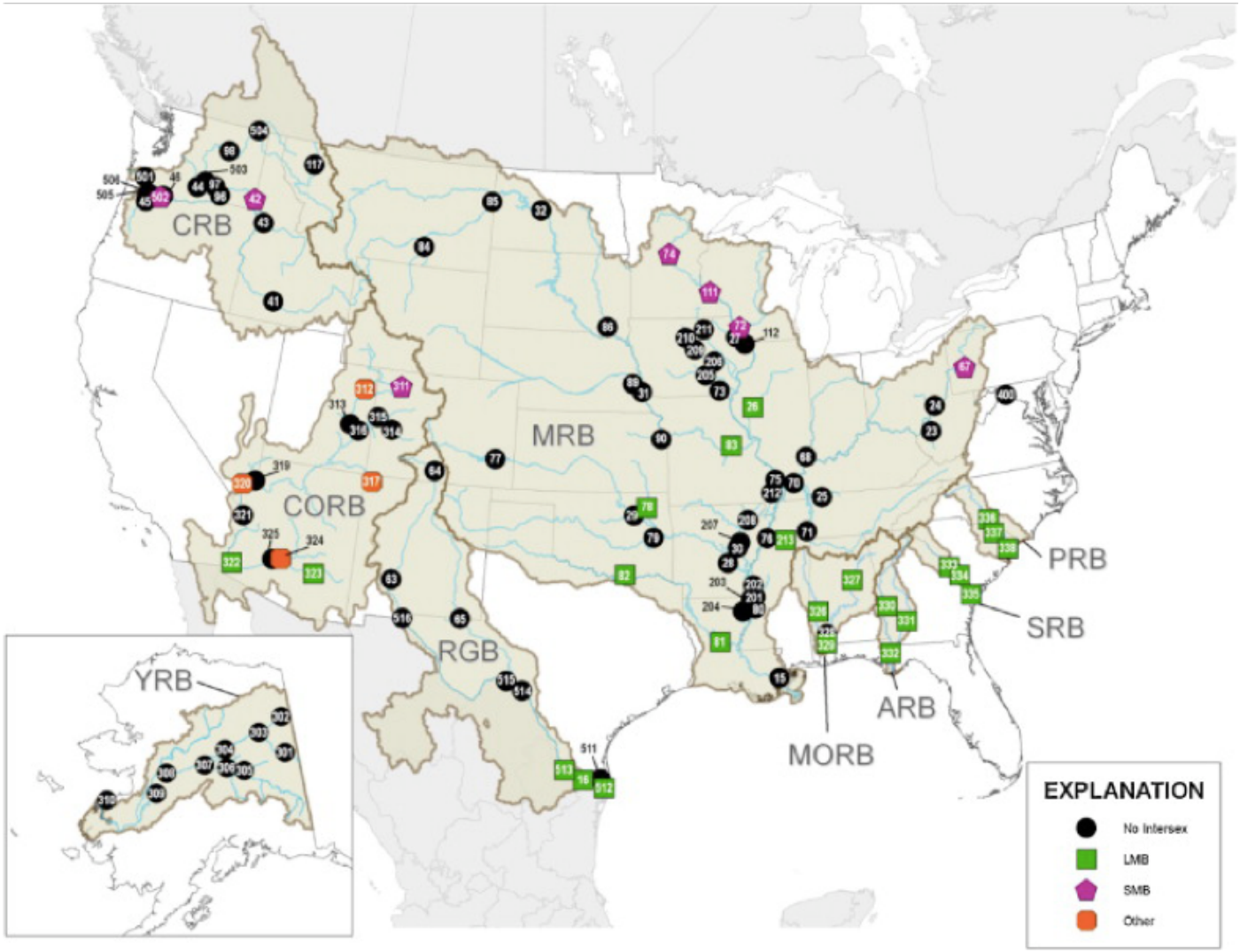


Coumestrol

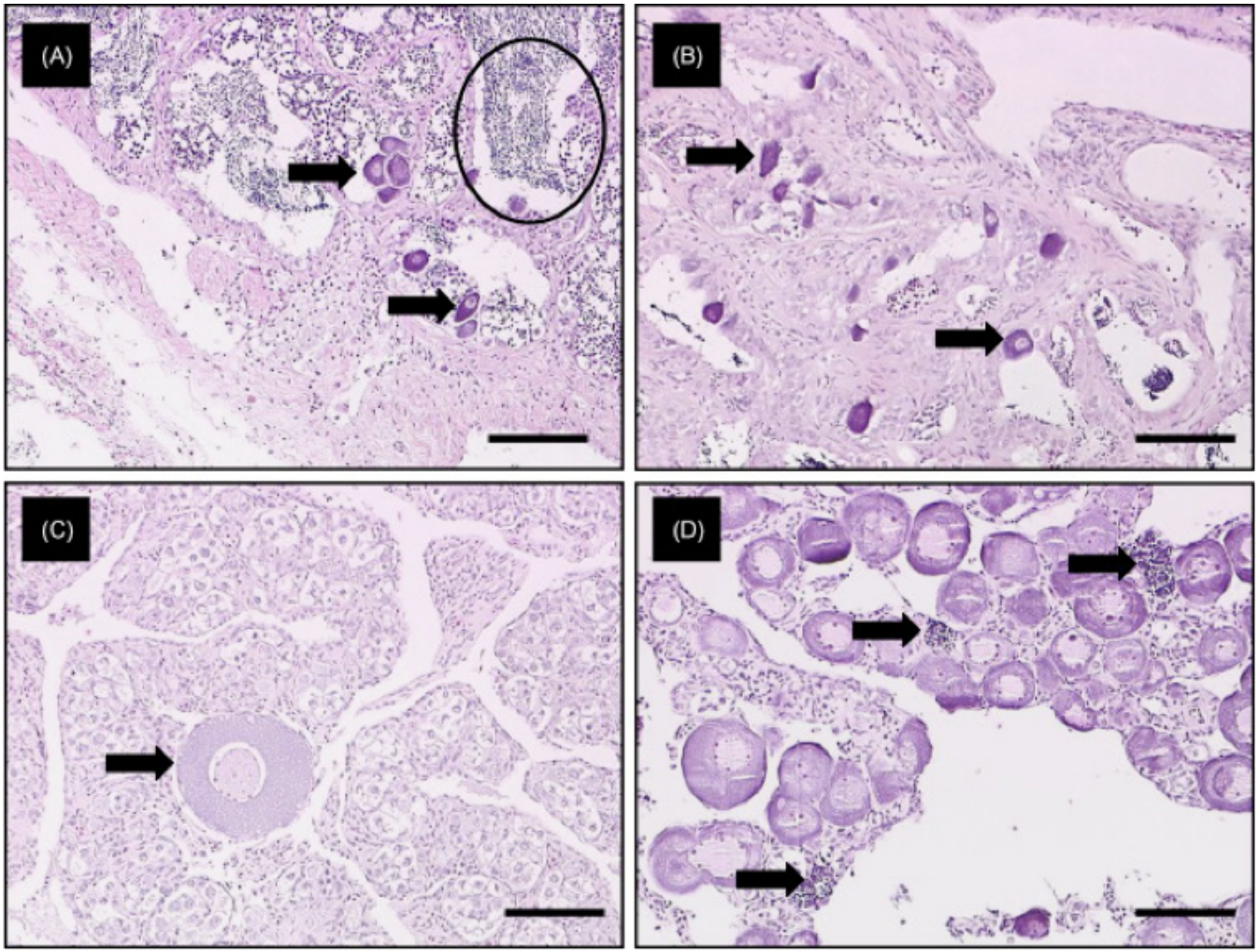


Genistein

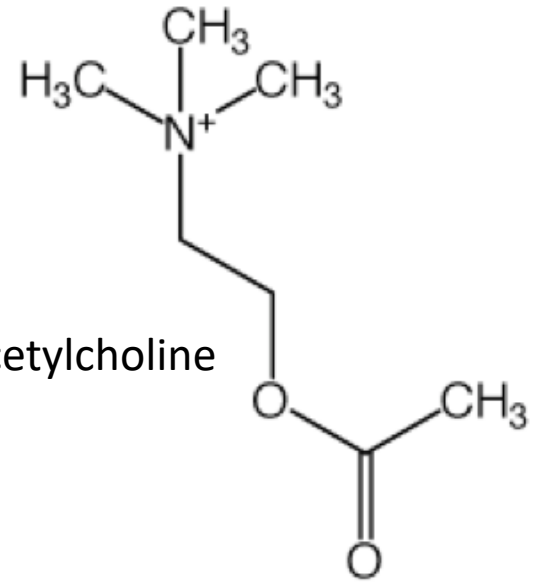
Aquatic Toxicology, Volume 95, Issue 1, 19 October 2009, Pages 60-70
Widespread occurrence of intersex in black basses (Micropterus spp.) from U.S. rivers, 1995–2004
Jo Ellen Hincka,et al.



Ovo-testis in male bass (and other fish)

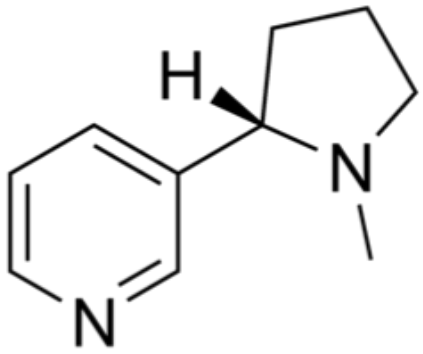


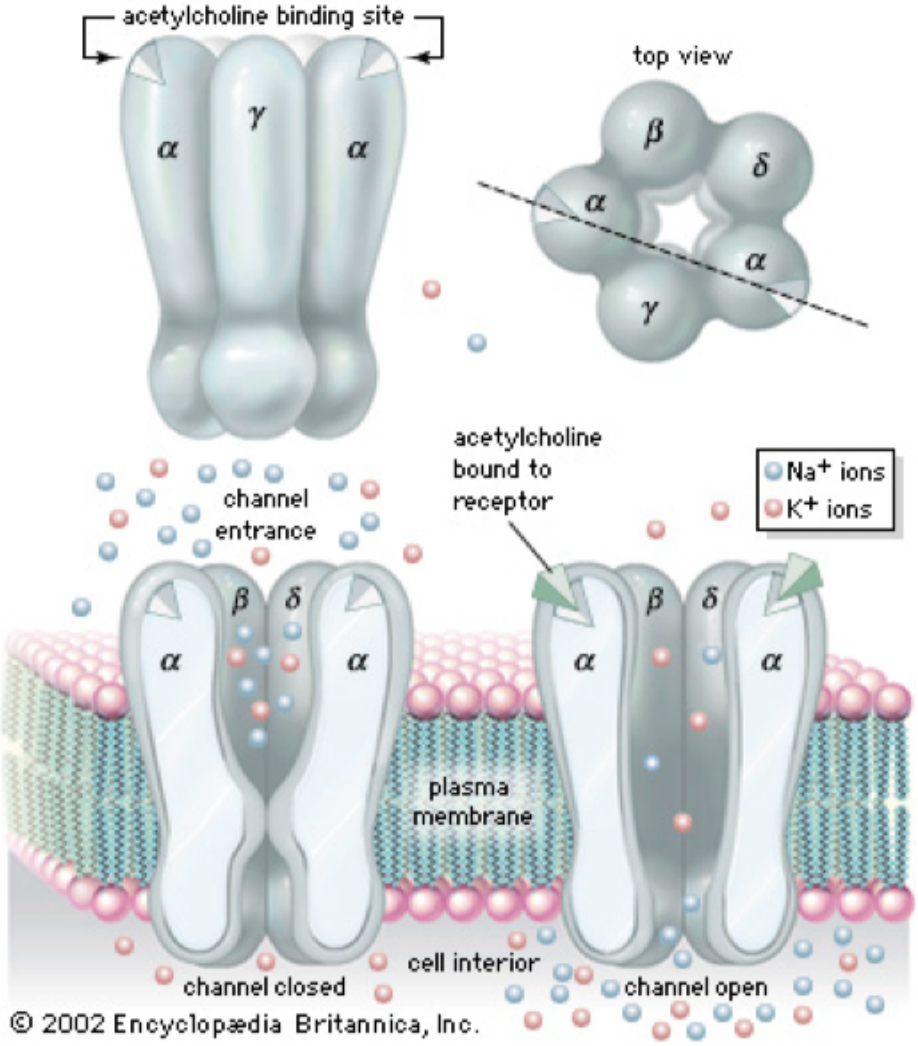
Acetylcholine "Nicotinic" Receptor



Acetylcholine

Nicotine





Review: Dose Response

- We use dose response information extensively to study drugs and other chemicals
- The dose is the most critical factor for a response
- In general, most chemicals display monotonic (sigmoidal) dose response curves when one endpoint is being measured
- Non-linear dose responses usually reflect multiple toxic endpoints (different targets/mechanisms)

Targets and Mechanisms

- The chemical dose must be sufficient in concentration to interact with the receptor/target – this is a basic principle of pharmacology and toxicology that is often ignored.
- Probably any biological molecule can be a target. The target is acted upon (bound, modified, activated, inactivated, etc.) by a chemical.
- Target refers to the site or particular molecule where the chemical interacts in some way. Target and receptor are somewhat synonymous terms. In general, the term receptor refers to an actual physiological receptor (e.g., estrogen receptor). Tubulin could be a non-physiological example of a receptor/target for Hg^{+2} (mercury ion).
- The mechanism refers to what the chemical does and how it does it.
- Targets and mechanisms change with dose.
- Knowing mechanisms and targets that are affected by low (relevant, real life) doses are the important ones.