STEP 2-REACTION OF THE ULTIMATE TOXICANT WITH THE TARGET MOLECULE

- ✓ Toxicity is typically mediated by a reaction of the ultimate toxicant a target molecule
- ✓ Subsequently, a series secondary biochemical events occur, leading to dysfunction or that is manifest at various levels of biological organization,

Such as at the target molecules itself cell organelles, cells, tissues, organs, and even the whole organism. Because interaction of the ultimate toxicant with the target molecule triggers the toxic effect, consideration is given to
(1) the attributes of target molecules,
(2) the types of reactions between ultimate toxicants and target molecules,
(3) the effects of toxicants on the target molecules
Finally consideration is given to toxicities that are initiated not by reaction of the ultimate toxicant with target molecules, but rather by alteration of the biological (micro)environment in which critical endogenous molecules, cell organelles, cells, and organs operate.

Attributes of Target Molecules

- ➢ Practically all endogenous compounds are potential targets for toxicants.
- ➢ The identification and characteristics of the target

molecules involved in toxicity constitute a major research priority, but a comprehensive inventory of potential target molecules is impossible.

- Nevertheless, the most prevalent and toxicologically relevant targets are macromolecules such as nucleic acids (especially DNA) and proteins.
- Among the small molecules, membrane lipids are frequently involved, whereas cofactors such as coenzyme A and pyridoxal rarely are involved.to be a target ,an endogenous molecule must possess the appropriate reactivity and/or steric configuration to allow the ultimate toxicant to enter into covalent or noncovalent reactions.
- For these reactions to occur, the target molecule must be accessible to a sufficiently high concentration of the ultimate toxicant. Thus, endogenous molecules that are exposed to reactive chemicals or are adjacent to sites where reactive metabolites are formed are frequently targets. Technical advances in. the field of proteonomics make it increasingly possible to identify potential protein targets of reactive chemicals as chemical-protein adducts. The first target for reactive metabolites is often the enzyme that catalyzes their production or the adjacent intracellular structures, For example, thyroperoxidase, the enzyme involved

in thyroid hormone synthesis, converts some nucleophilic xenobiotics into reactive free radicals that inactivate thyroperoxidase.This is the basis for the antithyroid as well as the thyroid tumor-inducing effect of these chemicals.

Thus, to conclusively identify a target molecule as being responsible for toxicity, it should be demonstrated that the ultimate toxicant

(1) reacts with the target and adversely affects its function,

(2) reaches an effective concentration at the target site, and

(3) alters the target in a way that is mechanistically related to the observed toxicity.

Types of Reactions

The ultimate toxicant may bind to the target molecules

- ✓ noncovalently or
- ✓ covalently and may alter it by
- ✓ hydrogen abstraction,
- ✓ electron transfer, or
- ✓ enzymatically.

Noncovalent Binding ;

- This type of binding can be due to apolar interactions or the formation of hydrogen and ionic bonds and is' typically involved in the interaction of toxicants with targets such as
- membrane receptors,
- intracellular receptors,
- ion channels, and some
- enzymes.

✓ For example, such interactions are responsible for the binding of strychnine to the glycine receptor on motor neurons in the spinal cord,

✓ warfarin to vitamin K 2,3-epoxide reductase.

✓ Such forces also are responsible for the intercalation of chemicals such as acridine yellow and doxorubicin into the double helix of DNA. These chemicals are toxic because the steric arrangement of their atoms allows them to combine with complementary sites on the endogenous molecule more or less as a key fits into a lock.

✓ Noncovalent binding usually is reversible

because of the comparatively low bonding energy.

Covalent Binding

- ❖Being practically irreversible, covalent binding is of great toxicologic importance because it permanently alters endogenous molecules
- ❖Covalent adduct formation is common with electrophilic toxicants such as nonionic and cationic electrophiles and radical cations.
- ❖These toxicants react with nucleophilic atoms that are abundant in biological macromolecules, such as proteins and nucleic acids.
- ❖Electrophilic atoms exhibit some selectivity toward nucleophilic atoms, depending on their charge-to-radius ratio.
- ❖In general, soft electrophiles prefer to react with soft nucleophiles (low charge-to-radius ratio in both), whereas
- ❖hard electrophiles react more readily with hard nucleophiles (high charge-to-radius ratio in both).

- ❖Metal ions such as silver and mercury are also

classified as soft electrophiles. (especially thiol groups).

- ❖ Conversely, hard electrophiles such as lithium, calcium, and barium, react preferentially as cations with hard nucleophiles (e.g., carboxylate and phosphate anions).

- ❖ Metals falling between these two extremes, such as chromium, zinc, and lead, exhibit universal reactivity with nucleophiles.

- ❖ The reactivity of an electrophile determines which endogenous nucleophiles can react with it and become a target.

Hydrogen Abstraction

- ➢ Neutral free radicals, such as those generated in reactions, can readily abstract H atoms from endogenous compounds, converting those compounds into radicals.
- ➢ Abstraction of hydrogen from thiols (R-SH) creates thiy radicals (R-S·), which upon radical recombination with **HO·** form sulfenic acids (R-S-

OH) that are precursors of disulfides (R-S-S-R)

- Radicals can remove hydrogen from CH2 groups of free amino acids or from amino acid residues in proteins and convert them to carbonyls.
- These carbonyls react covalently with amines, forming cross-links with DNA or other proteins. Hydrogen N02 is an oxidizing and nitrating species generated from ONOO- In addition, N02 is a contaminant in cigarette smoke, exhaust of gas engines and stoves, as well as the causative agent of "silo-filler's disease." is an occupational lung disease associated with toxic gas inhalation. The disease results from inhalation of nitrogen dioxide (NO_2) gas Often the gas penetrates throughout the lung and if severe can manifest as a form of Acute Respiratory Distress Syndrome,
- abstraction from deoxyribose in DNA yields the C-4'-radical,

Electron Transfer

- Chemicals can oxidize Fe(II) in hemoglobin to Fe(III), producing methemoglobinemia. is a disorder characterized by the presence of a higher than normal level of methemoglobin i.e., ferric [Fe^{3+}] rather than ferrous [Fe^{2+}] haemoglobin) in the blood

Enzymatic Reactions

- A few toxins act enzymatically on specific target proteins.
- For example, the plant toxins ricin and abrin are *N* -glycosidases; they hydrolyse a specific glycosidic bond in ribosomal RNS, blocking protein synthesis.
- Botulinum toxin acts as a Zn-protease; it hydrolyses the fusion proteins that assist in exocytosis of the neurotransmitter acetylcholine in cholinergic neurons, most importantly motoneurons, causing paralysis.
- The lethal factor component of anthrax toxin is also a Zn-protease, which inactivates mitogen-activated protein kinase kinase (MAPKK), inducing cell death.
- Other bacterial toxins catalyze the transfer for ADP-ribose from NAD+ to specific proteins
- Snake venoms contain hydrolytic enzymes that destroy biomolecules.
- In summary, most ultimate toxicants act on endogenous molecules on the basis of their chemical reactivity. Those with more than one type of reactivity may react by different mechanisms with various target molecules.
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Effects of Toxicants on Target Molecules

Reaction of the ultimate toxicant with endogenous molecules may cause dysfunction or destruction; in the case of proteins;

Dysfunction of Target Molecules

- Some toxicants activate protein target molecules, mimicking endogenous molecules.
- For example, morphine (pains) activates opioid receptors(in CNS),
- More commonly, chemicals inhibit the function of target molecules. Several xenobiotics such as atropinen block neurotransmitter receptors by interfering with the function of ion channels.
- Tetrodotoxin and saxitoxin, for example, inhibit opening of the voltage-activated sodium channels in the neuronal membrane,
- whereas DDT and the pyrethroid insecticides inhibit their closure.
- Chemicals that bind to tubulin or actin (e.g., impair the assembly (polymerization; formation of long chains) and/or disassembly (depolymerization) of these cytoskeletal proteins.
- Protein function is impaired when conformation or structure is altered by interaction with the toxicant.

Destruction of Target Molecules

In addition to adduct formation, toxicants alter the primary structure of endogenous molecules by means of cross-linking and fragmentation.

- Bifunctional electrophiles,such as 2,5-hexanedione, carbon disulfide, acrolein, cross-link cytoskeletal proteins, DNA, or DNA with proteins.
- HOOH and hydroxyl radicals can also induce cross-linking by converting proteins into either reactive electrophiles (e.g., protein sulfenic acids and protein carbonyls, respectively), which react with a nucleophilic group (e.g., thiol, amine) in another macromolecule.
- Radicals may induce cross-linking of macromolecules by converting them into radicals, which react with each other by radical recombination.
- Cross-linking imposes both structural and functional constraints on the linked molecules.
- Some target molecules are susceptible to spontaneous degradation after chemical attack.

These substances can readily react with adjacent molecules, such as membrane proteins, or diffuse to more distant molecules such as DNA.

NEOANTIGENIC FORMATION

An antigen is a foreign substance which is capable of inducing the production of specific antibodies, which react specifically in the body .

A Protein can undergo further modification within a biochemical pathway such as glycosylation, phosphorylation or proteolysis. This, by altering the structure of the protein, can produce new **neoantigenic determinants** as they give rise to new and require separate, specific antibodies for recognition