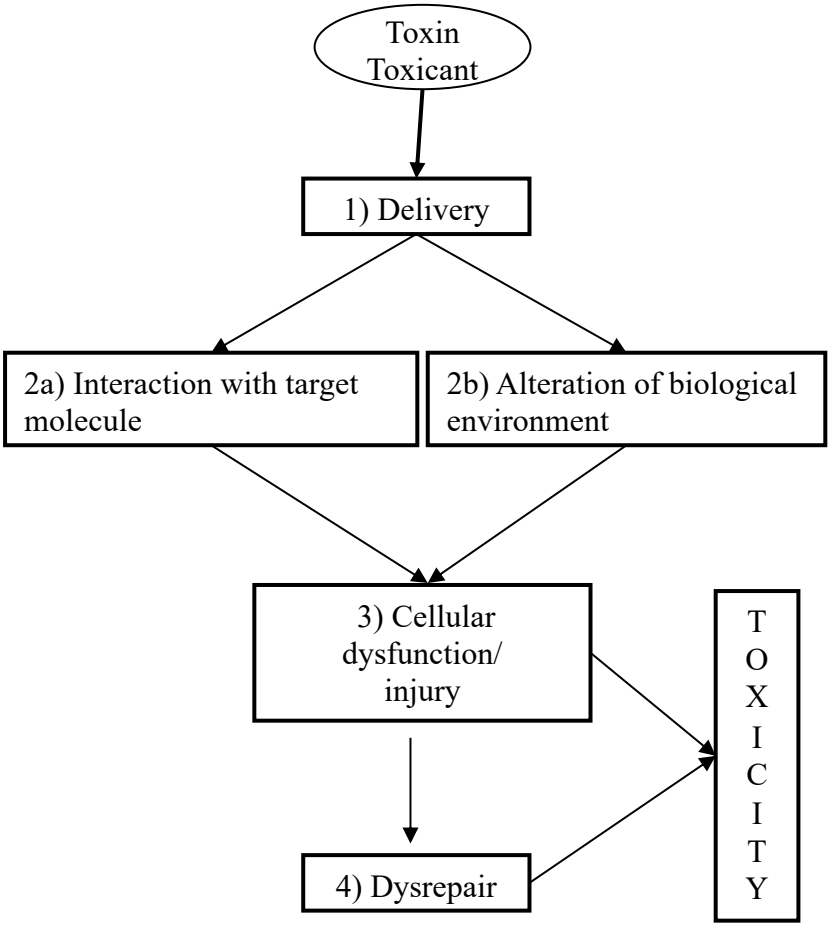
MECHANISMS OF TOXICITY



Adapted from Casarett & Doull's Toxicology. The Basic Science of Poisons. Ed. Klaassen, Curtis D.

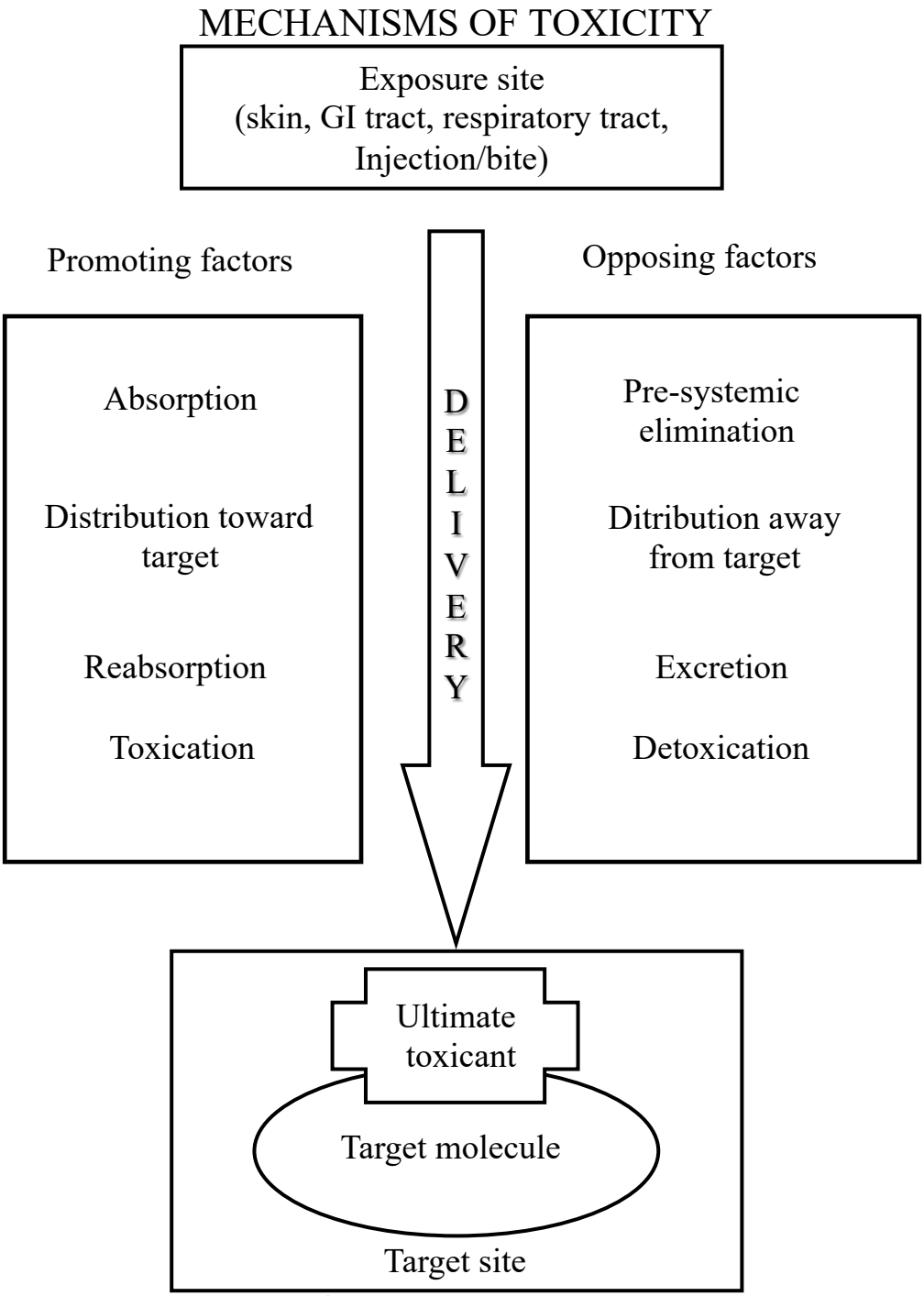
This scheme summarizes the main steps that lead to toxic effects following the exposure to a xenobiotic. The first step is the delivery of the xenobiotic inside the body (1). Once inside, the effects of the xenobiotic can be mediated by (2a) interactions with endogenous specific targets (e.g. receptors, enzymes, channels, etc.) or by (2b) alterations of the biological environment (e.g. changes in pH, precipitation in renal tubules, mitochondrial proton gradient collapse, etc.). At the cellular level, these effects can induce either cellular dysfunction, which means that the cell does not work properly, or a cellular injury, which means that part(s) of the cell are damaged.

Here are some examples of xenobiotics inducing toxic effects due to cell dysfunctions.

- Tetrodotoxin (TTX) is a very potent marine biotoxin present, for instance, in the skin, liver, ovary and viscera of puffer fish (fugu sashimi). This toxin is able to block some subtypes of voltage-dependent sodium channels causing reduction/blockade of impulse transmission especially in the peripheral nervous system. TTX intoxication leads to several symptoms in a dose-dependent manner and can be fatal by respiratory arrest due to muscle paralysis. At high doses, also heart failure can occur. There is no antidote and the only treatment is respiratory support and supportive care until TTX is eliminated.
- Sarin (O-isopropyl methylphosphonofluoridate) is a nerve agent (gas) used as a chemical weapon. Sarin is a potent organophosphorus agent that irreversibly inhibit acetylcholinesterase, the enzyme responsible for acetylcholine hydrolysis into choline and acetic acid. Therefore, its inhibition causes a pathologic build up of acetylcholine in the body leading to hyperstimulation of both muscarinic and nicotinic receptors (cholinergic crisis: miosis, blurred vision, sialorrhea, nausea, vomiting and diarrhea, bronchoconstriction and bronchorrhea, bradycardia) that can be fatal mainly due to flaccid paralysis and respiratory arrest. In this case, the patient can be treated with atropine to antagonize the muscarinic effects and with an oxime (e.g. pralidoxime) that is able to bind the organophosphorus agent and displace it from the enzyme.

- Intoxication with atropine (accidental or suicidal). As said before, atropine is an competitive antagonist of muscarinic cholinergic receptors, Therefore, intoxication with this drugs will result in symptoms that are due the blockade of normal acetylcholine effects mediated by these receptors (e.g. xerostomia, dilated pupils, blurred vision, flushing, nausea, confusion, hallucinations, tachycardia etc) and can be fatal (respiratory failure and circulatory collapse). Antidote is physostigmine, an acetylcholinesterase reversible inhibitor, or pilocarpine, a muscarinic receptor agonist.
- Intoxication with muscarine that is present in some types of mushrooms (in particular the *Inocybe* e *Clytocibe* species). It is a selective agonist of the cholinergic muscarinic receptors and can induce a variety of both peripheral (miosis and blurred vision, sialorrhea, sweating, bronchoconstriction with dyspnea and asthmatic attacks, bradycardia, vasodilation and hypotension, abdominal cramping, diarrhea) and central (tremors, convulsions, hypothermia) symptoms. Intoxication can be fatal if not treated with the antidote that is atropine.

On the other hand, xenobiotic interactions with many targets can induce cell injury by different mechanisms and toxicity manifests when damage exceed the cell detoxication/repair capacity and/or when repair mechanisms become malfunctional themselves. In general, such effects can lead to cell death and tissue necrosis/fibrosis or to genetic alterations leading, for example, to cancer or genetic disorders.



Adapted from Casarett & Doull's Toxicology. The Basic Science of Poisons. Ed. Klaassen, Curtis D.

The first step is **Delivery** that represents the movement of the xenobiotic from the site of exposure to the site where it will exert its toxic effect(s). This cartoon illustrates all the processes that can promote (left) or oppose (right) delivery and which are part of toxicokinetics (ADME, Absorbtion, Distribution, Metabolism and Elimination).

In most situations, the "ultimate toxicant" is the original xenobiotic (parent compound) to which a person has been exposed, but in other cases it is a metabolite of the parent compound or reactive oxygen or nitrogen species (ROS RNS) generated by the metabolism of the xenobiotic. Sometimes, the ultimate toxicant is an endogenous compound whose concentration raises well above its physiological levels (e.g. glutamate in the CNS).

Main factors influencing absorption

- Exposure frequency
- Xenobiotic physicochemical properties (partition coefficient)
- pH
- Surface area of absorption
- Characteristics of epithelium
- Vascularization
- P glycoprotein (P-gp)

Main factors influencing distribution

- Plasma protein binding
- Blood flow (high, medium and low perfused tissues)
- Endothelium structure of blood vessels (e.g. w/wo basal lamina, fenestrations, gap/tight junctions, barriers like blood brain barrier BBB)
- Specialized transports (e.g. P-gp on endothelial cells and on other cell membrane such as cardiomyocytes, kidney tubular cells; ion channels Pb and Ba enter cells via voltage-dependent calcium channels VDCCs, MPP+ enters dopaminergic neurons via dopamine transporters DAT)

Main factors influencing excretion

- Renal elimination (glomerular ultrafiltration, tubular secretion and tubular reabsorption; pH, P-gp)
- Hepatic elimination (biliary excretion and enterohepatic circulation)

TOXICATION

A large number of chemicals are directly toxic whereas in other cases toxic effects are maily mediated by metabolites of the parent compound. Such biotransformation process is called **toxication**.

Basically, there are 3 main types of toxication processes:

➢ Change of physicochemical properties of the xenobiotic leading to alteration of the microenvironment (e.g. ethylene glycol ⇒ oxalic acid ⇒ acidosis. In addition, in the presence of calcium, oxalic acid can precipitate as calcium oxalate in renal tubules).

➢Structural modifications permitting an efficient interaction with enzymes, receptors, etc. (e.g. parathion ⇒ paraoxon ⇒ inhibition of acetylcholinesterases; fluoroacetate ⇒ fluorocitrate ⇒ inhibition of aconitase ⇒ blocking of Krebs cycle)

➢ Structural modifications making a xenobiotic indiscriminately reactive toward endogenous molecules with susceptible functional groups.

A) Formation of electrophiles. Molecules with an electron-deficient atom with a full or partial charge (e.g. aldehydes, epoxides)

B) Formation of nucleophiles. Molecules that are electron-rich. Uncommon. (e.g. cyanide from amygdalin in cherry pits, apricot/apple seeds by bacterial β-glucosidase)

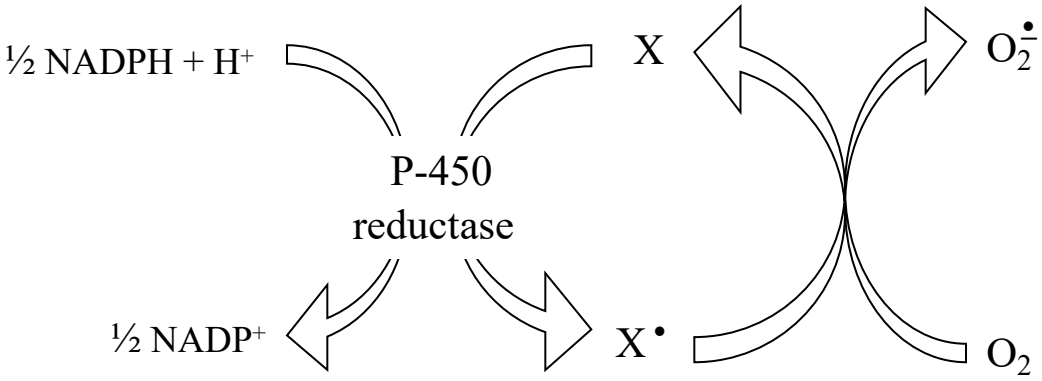
C) Formation of redox active reactants (e.g. reduction of nitrate to nitrite by intestine bacteria ⇒oxidation of hemoglobin to methemoglobin).

D) Formation of free radicals. Molecule or molecular fragment containing one or more unpaired electrons in the outer orbital.

TOXICATION

FORMATION OF FREE RADICALS

• **Acquisition of an electron (reduction).** In general, a xenobiotic accepts an electron (e.g. via NADPH cytochrome P450 reductase) and becomes a xenobiotic radical. Then, this radical transfers the extra electron to O_2 to form $O_2^{\bullet -}$ *(superoxide radical)* and regenerating the parent xenobiotic that can undergo again the same reaction. Therefore, one molecule of xenobiotic can generate many molecules of superoxide radical.

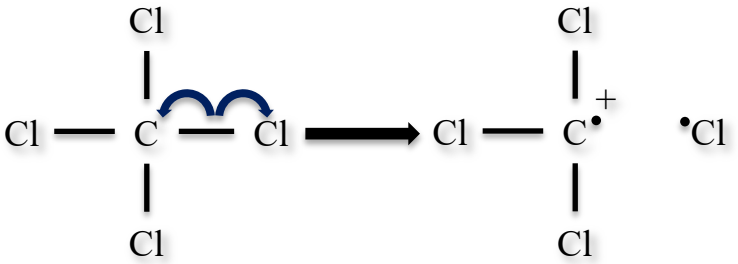


Adapted from Casarett & Doull's Toxicology. The Basic Science of Poisons. Ed. Klaassen, Curtis D.

• **Loss of an electron (oxidation).** Nucleophiles (phenols, amines, thiols) can lose an electron (e.g. during peroxidase or cytochrome P450-mediated reactions).

• **Homolytic fission of a covalent bond.** Reductive fission triggered by electron transfer for instance from cytochrome P450 or mETC.

$CCl_4 \Rightarrow {}^{\bullet}CCl_3 + Cl^{\bullet}$ (carbon tetrachloride ⇒ trichloromethyl radical + chlorine radical)



${}^{\bullet}CCl_3 + O_2 \Rightarrow Cl_3COO^{\bullet}$ (trichloromethylperoxyl radical)

SUPEROXIDE RADICAL

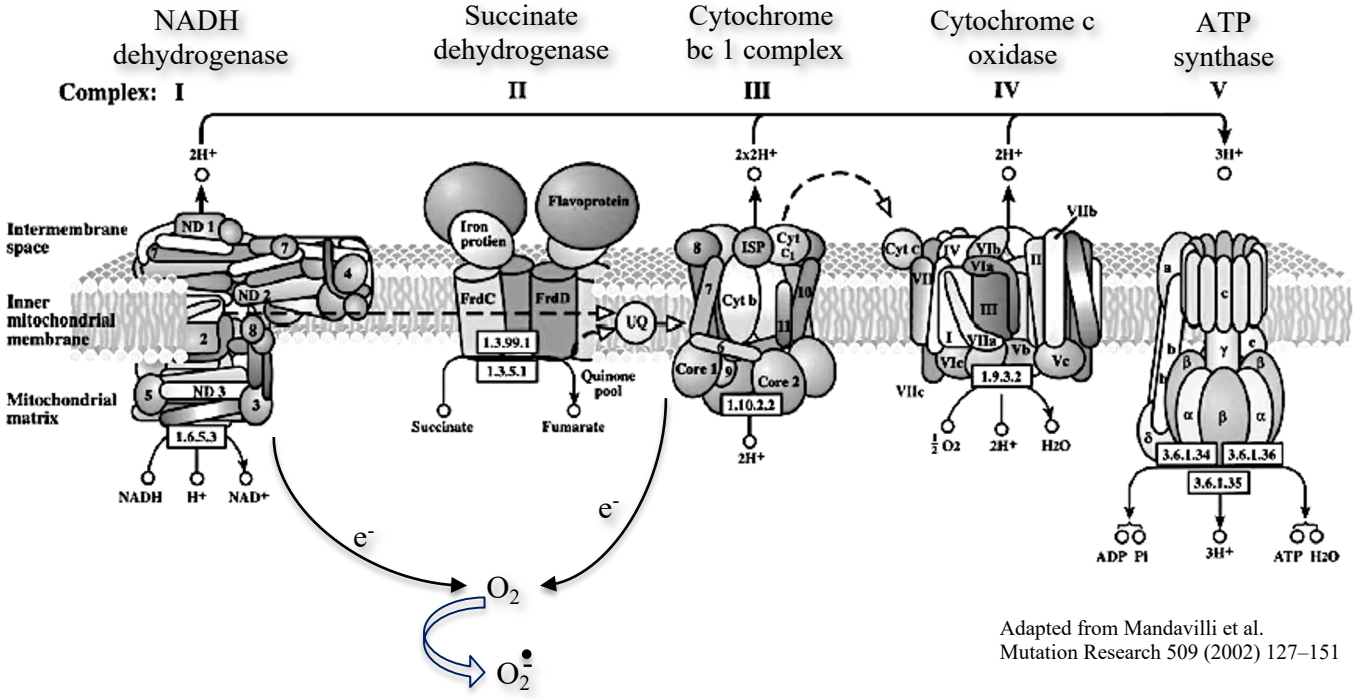
Other biosynthetic routes

Superoxide radicals can be also generated by different physiological reactions

1. Mitochondrial electron transport chain
2. NADPH Oxidases
3. Ciclooxygenases and Lipoxygenases
4. Xanthine oxidase
5. Auto-oxidation of endogenous molecules

1. Mitochondrial electron transport chain (mETC)

During oxidative phosphorylation, there is a leakage of electrons from the mETC, in particular from Complex I and Coenzyme Q/Complex III. As a rule of thumb, every 20 molecules of O_2 that enter the mETC, one molecule of superoxide radical is formed.



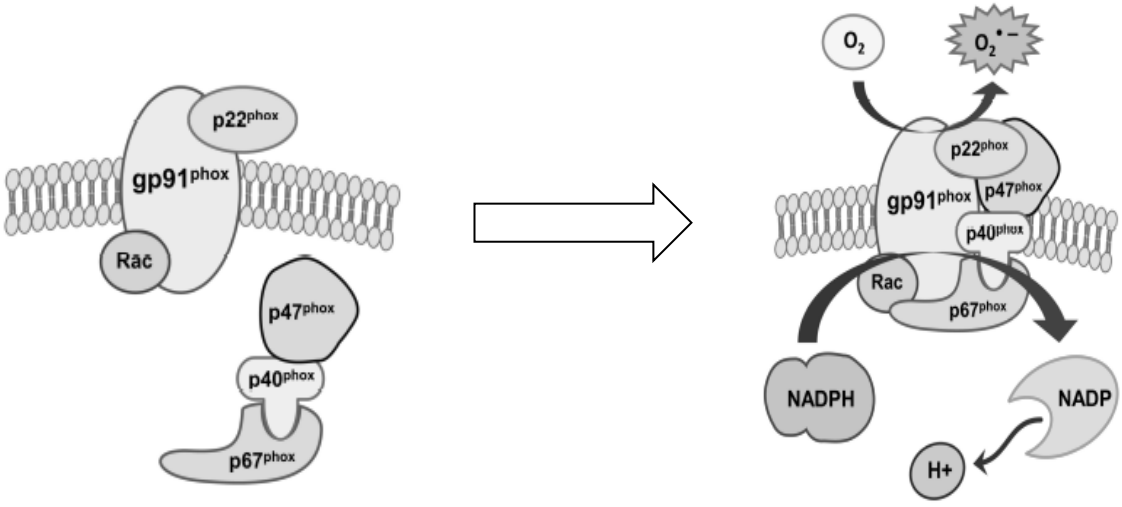
Adapted from Mandavilli et al.
Mutation Research 509 (2002) 127–151

2. NADPH OXIDASES (NOXs)

• NOX
Five isoforms (NOX1-5)

• DUOX (Dual oxidases)
Two isoforms (DUOX1-2)

They comprise membrane-bound and cytosolic subunits. Upon activation, cytosolic subunits migrate to the membrane to dock with the membrane subunits.



Kim et al. (2017) Exp. Neurobiol. 26:195-205

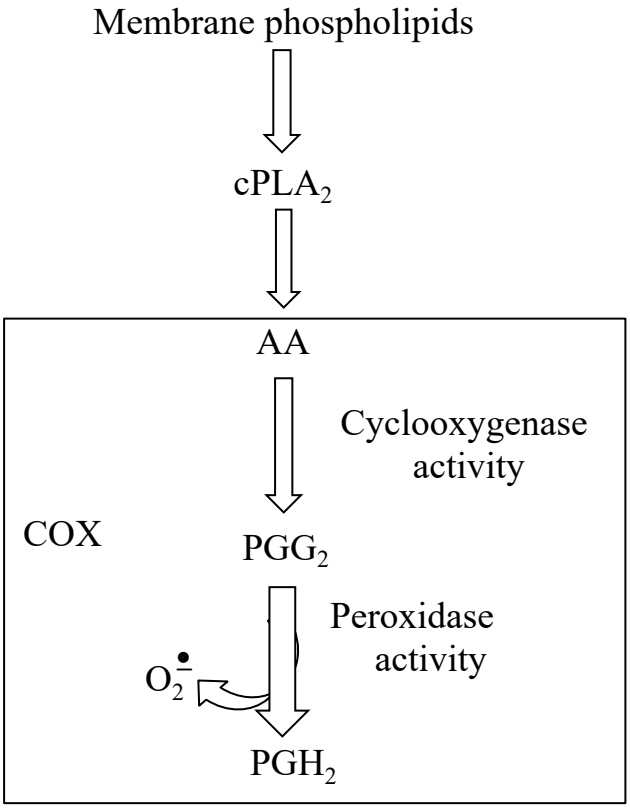
These multisubunit enzymes produce $O_2^{\bullet-}$ using NADPH.

The various isoforms have different distribution in species and tissues, in phagocytic and non phagocytic cells.

They are activated by many signals (e.g. Ang II, thrombin, PDGF, etc.)

3. COX Enzymes

Cyclooxygenases (COX) are a family of enzymes that comprises both constitutive (COX1) and inducible forms (COX-2). These enzymes possess a double enzymatic activity. Once arachidonic acid (AA) is produced from membrane phospholipids by the action of cytosolic phospholipase A_2 ($cPLA_2$), it is transformed by COX into prostaglandin G_2 (PGG_2) by a cyclooxygenase reaction. PGG2 is then reduced by peroxidase activity into prostaglandin H_2 (PGH_2). During this reaction, superoxide radicals, as well as carbon-centered radicals can be formed.



4. Xanthine oxidase

This enzyme can derive from the calcium-dependent proteolytic cleavage of xanthine dehydrogenase that can occur under certain conditions (e.g. ischemia). Both xanthine dehydrogenase and oxidase are involved in the metabolism of xanthinic bases but with a critical difference.

In fact, xanthine dehydrogenase (**XD**) uses NAD^+ to oxidize hypoxanthine to xanthine and xanthine to uric acid and there is no production of radicals. On the other hand, xanthine oxidase (**XO**) uses molecular oxygen for the same reaction and can produce superoxide radicals/hydrogen peroxide.

1. XD

Hypoxanthine $\xrightarrow{NAD^+ \; NADH}$ Xanthine $\xrightarrow{NAD^+ \; NADH}$ Uric acid

2. XO

Hypoxanthine $\xrightarrow{O_2 \; O_2^{\bullet-}/H_2O_2}$ Xanthine $\xrightarrow{O_2 \; O_2^{\bullet-}/H_2O_2}$ Uric acid

5. Auto-oxidation of endogenous molecules

Several endogenous molecules can undergo auto-oxidation in the presence of trace metals (e.g. iron, copper)

$$R\text{-}SH \xrightarrow{Fe/Cu + O_2} RS^{\bullet} + O_2^{\bullet -}$$

$$\text{Catecholamines} \xrightarrow{Fe/Cu + O_2} \text{semiquinones} + O_2^{\bullet -}$$

OTHER ENDOGENOUS FREE RADICALS

NITROGEN MONOXIDE (NITRIC OXIDE)

NO is a gaseous free radical of nitrogen. At low concentrations, it acts as a transmitter with autocrine or paracrine functions, whereas at high concentrations is cytotoxic.

NO-SYNTHASE (NOS)

NO is synthesised by NO-synthase (NOS), a family of enzymes containing both FMN/FAD that needs NADPH and tetrahydrobiopterin as co-factors. There are two different families of NOS.

COSTITUTIVE	INDUCIBLE
Ca^{2+}-calmodulin dependent enzymes	Ca^{2+}-indipendent enzymes
Active as dimer	Active as dimer
Produce pM-nM NO levels	Produce nM-µM NO levels
NEURONAL NOS (nNOS; NOS I) 160 kDa	MACROPHAGIC NOS (iNOS; NOS II) 130 kDa
ENDOTHELIAL NOS (eNOS; NOS III) 130 kDa	

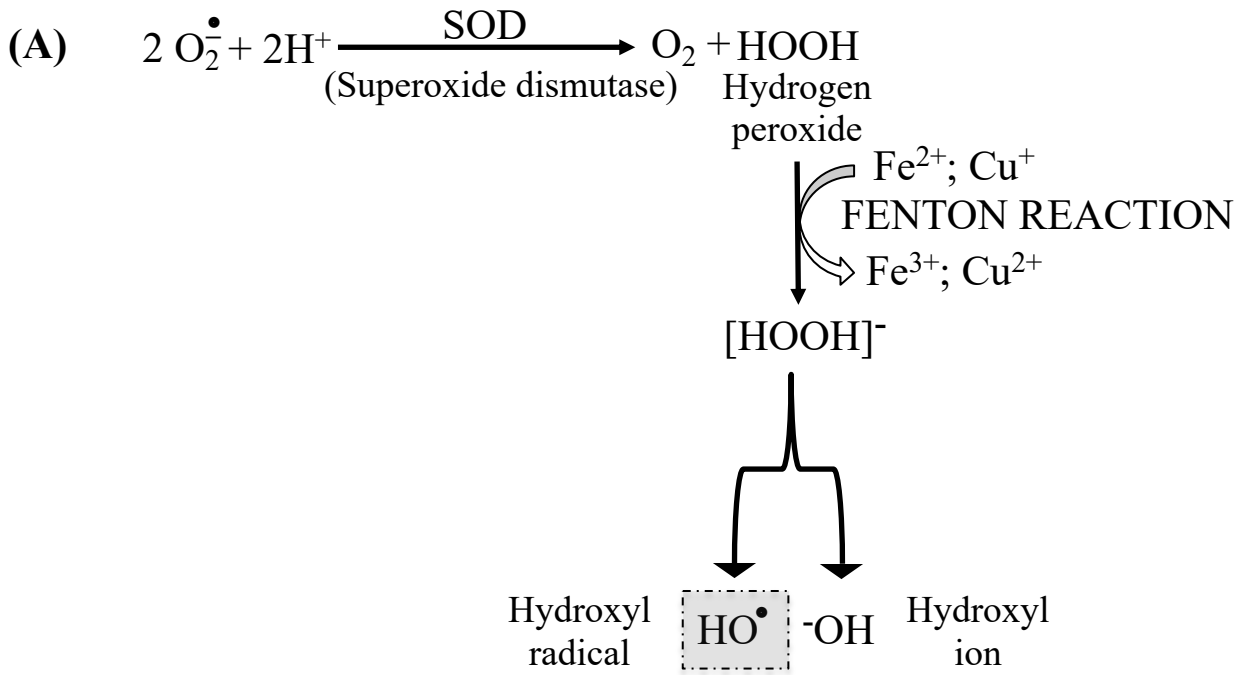
NO-Synthases produce NO by transforming l-arginine into l-citrulline with a 1:1:1 stoichiometry

$$1\ \text{l-Arg} + O_2 \xrightarrow{NOS} 1\ \text{l-Citr} + 1\ ^{\bullet}NO$$

ROS AND RNS:
OXIDATIVE AND NITROSATIVE STRESS

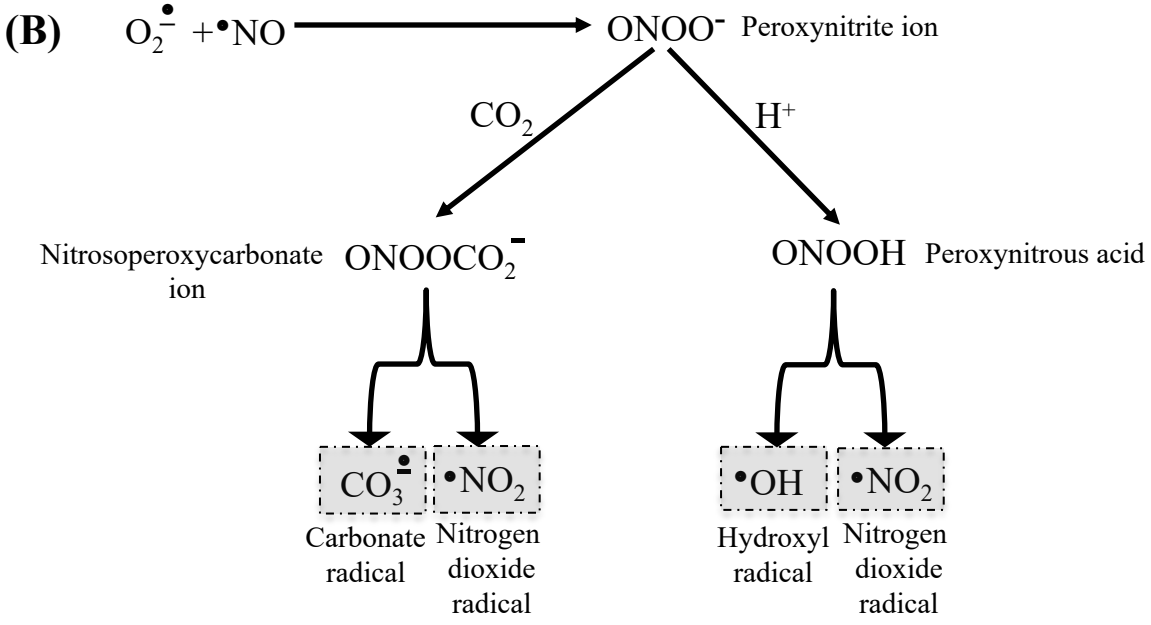
In the vast majority of cases, $O_2^{\bullet-}$ and $^{\bullet}NO$ are not the ultimate radicals responsible of cell injury. In fact, as shown in the figure below, they are transformed in more reactive radicals that, if accumulate, trigger oxidative and nitrosative stress.

In pathway (**A**), superoxide radicals are transformed into hydrogen peroxide by superoxide dismutase, a family of enzymes that are present in mitochondria, cytosol and the extracellular space. Actually, this represents the first reaction to detoxicate superoxide radicals. However, if hydrogen peroxide is not adequately detoxicated (see Detoxication), it accumulates and, in presence of trace metals (e.g. Fe^{2+} or Cu^{+}), can be transformed by Fenton reaction into hydroxyl ion and hydroxyl radical, the most reactive oxygen radical.



ROS AND RNS:
OXIDATIVE AND NITROSATIVE STRESS

In pathway **(B),** the superoxide radical reacts with nitrogen monoxide (in and around those cells that possess the different isoforms of NO-synthase) to form peroxynitrite ion. This ion can react with carbon dioxide to form nitrosoperoxycarbonate ion that can undergo spontaneous homolyzation to produce two reactive radicals: carbonate radical and nitrogen dioxide radical. Although still a matter of debate, peroxynitrite ion can also react with hydrogen ions to form peroxynitrous acid that can produce hydroxyl radical and nitrogen dioxide radical. Nitrogen dioxide radical, beside being an oxidant, it is also a nitrating agent causing the addition of a nitro group into organic molecules R-NO_2.



DETOXICATION

- Detoxication of toxicants with no functional groups. Xenobiotic with no functional groups (e.g benzene and toluene) are detoxicated by phase I reactions (functionalization) followed by phase II reactions (conjugation).

- Detoxication of nucleophiles. These compounds (e.g. R-OH, R-NH_2, R-SH) are detoxicated by phase II reactions with conjugation to the nucleophilic functional group. In some cases, they are also inactivated by oxidation.

- Detoxication of electrophiles. In the majority of cases, detoxication occurs by phase II conjugation with the thiol nucleophile glutathione (glu-cys-gly). Also hydrolysis, reduction and oxidation can occur.

- Detoxication of free radicals. Free radicals can be detoxicated by enzymatic and non-enzymatic reactions.

Enzymatic mechanisms

As shown before, many reactions lead to the formation of superoxide radical that is first transformed to hydrogen peroxide by superoxide dismutase **(A)**. Then, hydrogen peroxide is further metabolized to water by catalases in peroxisomes **(B)** or by glutathione peroxidase (GPO) in cytosol **(C)**. In this latter reaction, reduced glutathione (GSH) is transformed into oxidized glutathione (GSSG).

(A) $2\ O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$

(B) $2\ H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$

(C) $H_2O_2 + 2$ Glu---Cys---Gly(SH) (GSH) $\xrightarrow{GPO}$ Glu---Cys---Gly(S–S)Glu---Cys---Gly (GSSG) $+ 2\ H_2O$

Then, GSSG is reduced back to GSH by glutathione reductase (GR) that uses NADPH as cofactor **(D)**

(D) $GSSG + NADPH + H^+ \xrightarrow{GR} 2\ GSH + NADP^+$

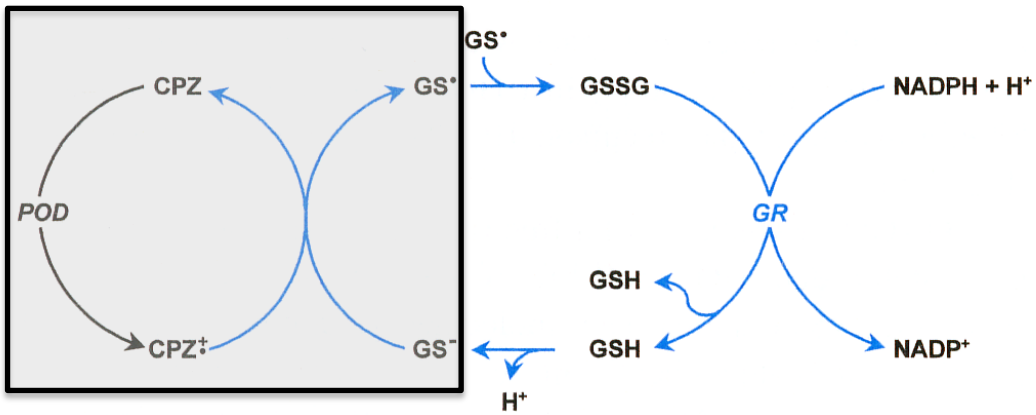
This antioxidant system is also able to detoxicate also alkyl hydroperoxides (R-OOH), which originate from alkyl peroxyl radicals (ROO•, e.g during lipid peroxidation), into alcohols (ROH).

DETOXICATION

$$ONOO^- \xrightarrow[\text{peroxidase}]{\text{Glutathione}} NO_2^-$$

Non enzymatic mechanisms

1. Reaction with glutathione



Adapted from Casarett & Doull's Toxicology. The Basic Science of Poisons. Ed. Klaassen, Curtis D.

2. Reaction with heme-containing proteins

$$^{\bullet}NO + oxyHb \longrightarrow metHb + NO_3^-$$

$$ONOO^- + oxyHb \longrightarrow metHb + NO_3^-$$

3. Reactions with ascorbic acid

$$AH_2 + O_2^{\bullet -} + H^+ \longrightarrow AH^{\bullet} + H_2O_2$$

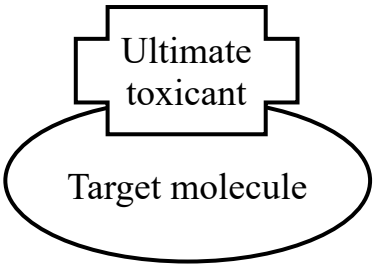
$$AH^{\bullet} + O_2^{\bullet -} + H^+ \longrightarrow A + H_2O_2$$

$$2\,AH^{\bullet} \longrightarrow A + AH_2$$

$$A + 2\,GSH \xrightarrow[\text{peroxidase}]{\text{Glutathione}} AH_2 + GSSG$$

$$AH^{\bullet} \xrightarrow{\text{Reduction}} AH_2$$

REACTION WITH THE TARGET MOLECULE



Target site

1. TARGET ATTRIBUTES

a) Appropriate reactivity. Of course, the presence of appropriate reactive functional groups in the cellular/molecular target with which the toxicant can easily react is one of the main factor influencing the toxic effect.

b) Accessibility. The molecular target must be accessible to a sufficiently high concentration of the toxicant for the toxic effect to occur. (e.g. MMP+ selectively kills nigrostriatal dopaminergic neurons because it enters into them through the dopamine transporter DAT and accumulates in them due to the presence of high amount of the pigment neuromelanin that has high affinity for MPP+)

c) Critical function of the target (e.g. CO binds to CYP450 and haemoglobin but the toxic effect is due to the interaction with latter target)

2. TYPES OF REACTIONS WITH THE TARGET

a) Hydrogen, ionic, dipolar bonds with membrane and cytosolic receptors, ion channels, enzymes, carriers, etc

b) Irreversible covalent bonds with permanent alterations (electrophiles and free radicals, e.g. $^{\bullet}OH$, $^{\bullet}NO_2$)

c) Hydrogen abstraction (free radicals). The radical can cause the homolytic cleavage of a R-H bond, thus abstracting a $^{\bullet}H$, which then binds to the radical, leaving one electron on the target molecule that becomes a radical.

1) Proteins. $R\text{-}SH + X^{\bullet} \Rightarrow R\text{-}S^{\bullet} + XH \Rightarrow R\text{-}S^{\bullet} + R\text{-}S^{\bullet} \Rightarrow R\text{-}S\text{-}S\text{-}R$

$R\text{-}CH_2\text{-}R + X^{\bullet} \Rightarrow R\text{-}{}^{\bullet}CH\text{-}R + XH \Rightarrow R\text{-}\overset{\overset{O}{\|}}{C}\text{-}R$ (protein carbonyls)

2) membrane lipids (lipid peroxidation)

3) DNA deoxyribose $\Rightarrow$ C-4′ radical $\Rightarrow$ DNA cleavage

d) Redox reactions. Oxidants (e.g. nitrites) can change $Fe^{2+} \Rightarrow Fe^{3+}$ (Hb $\Rightarrow$ metaHb)

e) Enzymatic reactions. Many natural toxins have enzymatic activity (e.g. proteases, phospholipases).

3. OUTCOMES.

In general, the interaction of the xenobiotic with its target can result in:

A) Target dysfunction

1) Activation/blockade of enzymes/receptors
2) Activation/blockade of ion channels
3) Blockade of transporters
4) Alterations of mitochondrial functions
5) Alterations of proteins or protein synthesis
6) Interactions with DNA leading to blockade/errors in replication/transcription

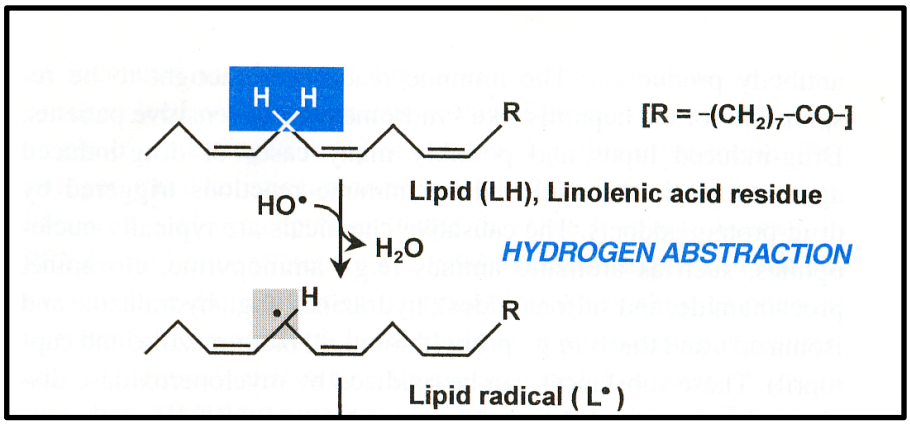
B) Target destruction

1) Cross-link and fragmentation of cystoscheletal proteins or DNA
2) Lipid peroxidation

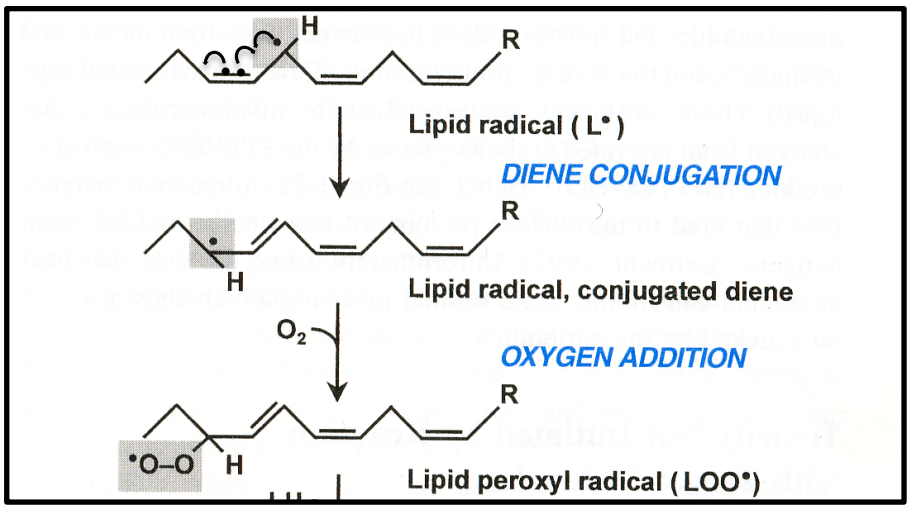
LIPID PEROXIDATION

Lipid peroxidation is the typical example of free radical-mediated damage to cell endogenous macromolecules resulting in their fragmentation.

This figure shows the attack by the hydroxyl radical to a reactive methylene group present in a residue of linolenic acid with hydrogen abstraction that leads to a carbon-centered lipid radical (L•)



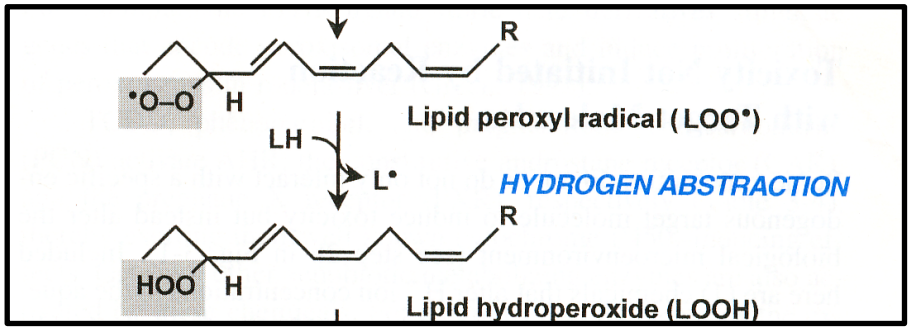
This lipid radical stabilizes by diene conjugation, thus forming a lipid radical, conjugated diene. This lipid radical then reacts with molecular oxygen to form a lipid peroxyl radical (LOO•).



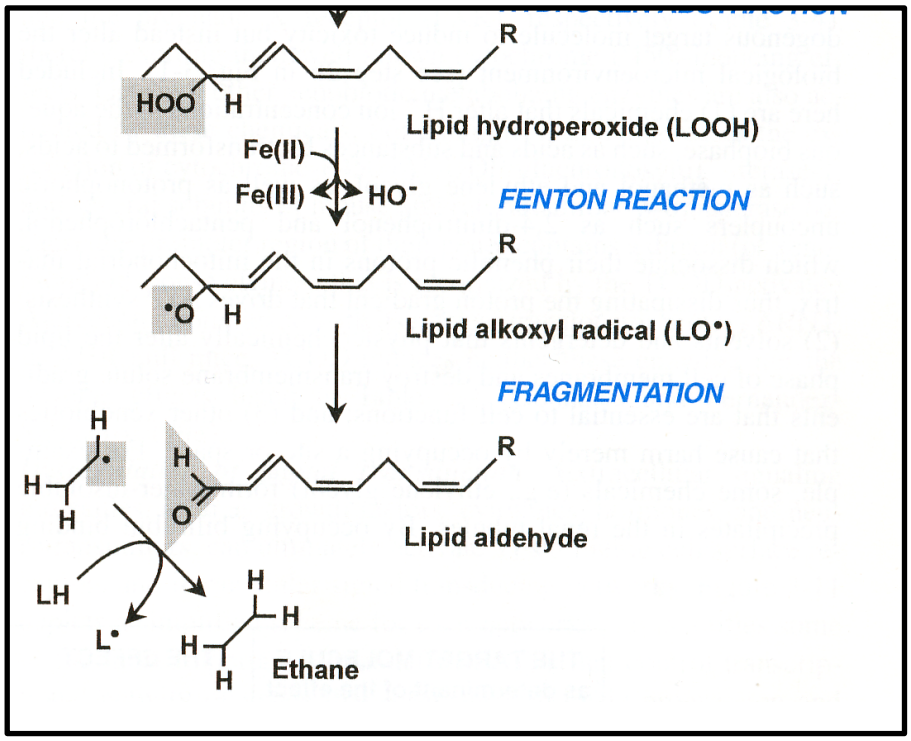
Adapted from Casarett & Doull's Toxicology. The Basic Science of Poisons. Ed. Klaassen, Curtis D.

LIPID PEROXIDATION

The lipid peroxyl radical (LOO•) abstracts a hydrogen atom from a methylene group of a nearby lipid (LH), exactly as shown at the beginning of the scheme, to become a lipid hydroperoxide (LOOH). The nearby lipid becomes a lipid radical (L•) that can undergo the reactions that have been shown so far, thus triggering radical chain reactions.



Like hydrogen peroxide (HOOH), the lipid hydroperoxide (LOOH) undergoes the Fenton reaction to form the hydroxyl ion ($^{-}$OH) and a lipid alkoxyl radical (LO•) that causes a fragmentation at the level of the last two carbon atoms with the formation of lipid aldehydes (e.g. malondialdehyde and 4-hydroxynonenal) and ethyl radical. This, in turn, can cause hydrogen abstraction from another lipid (LH) to form another lipid radical (L•)



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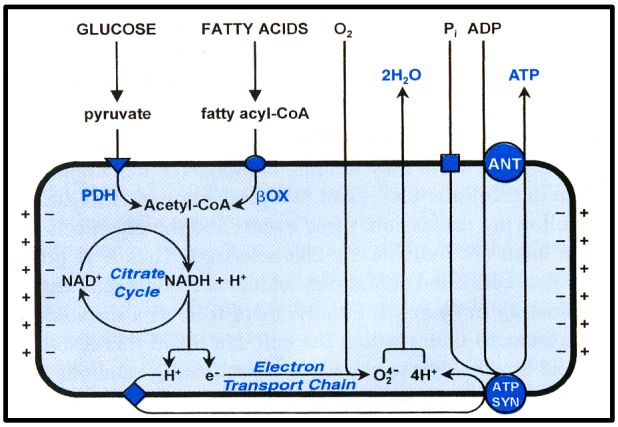
CELLULAR MECHANISMS CAUSING CELL DEATH

The main cellular mechanisms that can lead to cell death are:
1) ATP depletion due to mitochondrial toxicity
2) Alteration of calcium homeostasis
3) Oxidative/nitrosative stress

1) ATP depletion. ATP is the primary source of energy for a myriad of cellular processes (e.g. active transport, cell motility, cell muscle contraction, cell division, etc.), it is essential for a variety of phosphorylation reactions, it is part of many molecules (e.g. NAD(P)H). Many xenobiotics target mitochondria where they can alter the different biochemical steps leading to ATP synthesis.



Adapted from Casarett & Doull's Toxicology. The Basic Science of Poisons. Ed. Klaassen, Curtis D.

<u>Inhibitors of hydrogen delivery to the electron transport chain</u>
a) Inhibition of glycolysis (iodoacetate)
b) Inhibition of PDH (arsenite, benzoquinone)
c) Inhibition of Krebs/citrate cycle (fluoroacetate, ethanol, 3-nitropropionic acid)
d) NADH depletors (MPP+, menadione, fatty acid hydroperoxides)

<u>Inhibitors of electron transport</u>
a) Complex I (NADH dehydrogenase) inhibitors (paraquat, MPP+, rotenone)
b) Complex II (Succinate dehydrogenase) inhibitors (3-nitropropionic acid)
c) Complex III (Cytochrome bc1 complex) inhibitors (antimycin-A)
d) Complex IV (Cytochrome oxidase) inhibitors (cynade, azide, NO)
e) Multisite inhibitors (diphenylether erbicides)
f) Electron acceptors (CCl_4, MPP+)

<u>Inhibitors of oxygen delivery</u>
a) Methemoglobin- (aniline dyes, NO_2^- , drugs) and carboxyhemoglobin-forming chemicals (CO)
b) Chemicals causing ischemia (ergot alkaloids, cocaine)
c) Chemicals depressing respiration (CNS depressants e.g. opioids)

Inhibitors of ADP phosphorylation

a) ADP transport inhibitors (DDT, free fatty acids)
b) Phosphate transport inhibitors (N-ethylmaleimide, mersalyl)
c) Membrane potential uncouplers (erbicides, salicylate)
d) ATP synthase inhibitors (DDT, oligomycin)

Chemicals causing damage to mitochondrial DNA

Antiviral drugs; chronic ethanol

CYANIDE

Cyanide ion (CN^-) has a high affinity for Fe^{3+}. The toxic effect is due to inhibition of mitochondrial cytochrome-c oxidase, disruption of the transport of electrons to molecular oxygen and blocking of ATP production. Toxic effect is therefore caused not by an insufficient availability of oxygen but by the impossibility of using it.

Sources of cyanide

- HCN (hydrogen cyanide) and C2N2 (cyanogen) used as fumigants for disinfestation
- KCN industrial and artisanal use, fumigant
- Cyanide solutions are used for gold and silver extraction, photographic development processes, steel hardening processes, plastic production, etc.
- Cyanide is also released during coal combustion, burning of plastics or household waste, cigarette smoking, etc.
- Amygdalin is a cyanogenic glycoside present in seeds/kernels of apricots, cherries, plums, bitter almonds, apples, peaches. Following ingestion, amygdalin is transformed by intestinal enzymes into HCN and benzaldehyde.

Acute intoxication: hot flashes, headache, tachypnea, dizziness, tachycardia and bradycardia, coma, convulsions and death.
Chronic intoxication: headache, dizziness, nausea, vomiting, taste of bitter almonds, chest pain.

Treatment of poisoning

Amyl nitrite (inhalation) plus sodium nitrite (i.v.): this treatment transforms hemoglobin into methemoglobin (approx. 40%) that competes with cytochrome-c oxidase for binding cyanide with the formation of cyanmethemoglobin. Then, sodium thiosulfate ($Na_2S_2O_3$) can be administered to accelerate the dissociation of cyanide from cyanmethemoglobin by transforming unbound cyanide into sodium thiocyanate (NaSCN) that is eliminated in urine.

2) Alteration of calcium homeostasis

Calcium ions (Ca^{2+}) play a critical role in a vast number of cellular processes.

In general:
Intracellular $[Ca^{2+}] \approx 0.1$ μM
Extracellular $[Ca^{2+}] \approx 1000$ μM (1 mM)
This means that there is a great concentration gradient that drives Ca^{2+} inside cells. However, cell membranes are impermeable to this cation. Therefore, there are different mechanisms that regulate the increase of Ca^{2+} inside cells.

1) Ca^{2+} entry through voltage-operated calcium channels (VOCCs) or voltage-dependent calcium channels (VDCCs) in excitable cells. Low voltage activated (LVA) like T channels or high voltage activated (HVA) like L, N, P, Q and R.

2) Ca^{2+} entry through receptor-operated channels (ROC) that are ionotropic receptors present both on excitable and nonexcitable cells

3) Ca^{2+} release from intracellular stores such as the endoplasmic/sarcoplasmic reticulum following activation of IP3 receptors or ryanodine-sensitive receptors.

Cells have several mechanisms to control free calcium levels in the cytosol:

<u>Ca^{2+}-buffering mechanisms</u>
A) Calcium binding proteins.
Parvalbumin, calbindin that are just «buffer» proteins
Calmodulin, the prototype of «trigger» proteins that, following Ca^{2+} binding, activates different enzymes

B) Endoplasmic reticulum
It sequesters Ca^{2+} by low-affinity (Km 1-3 μM)/high capacity (high Tmax), ATP-dependent transporters

C) Mitochondria
They sequester Ca^{2+} by low-affinity (Km 2-10 μM)/high capacity transporters using mitochondrial membrane potential ($\Delta\Psi_m$) as energy.

D) Nucleus
It sequesters Ca^{2+} by low-affinity/high capacity, ATP-dependent transporters

<u>Extrusion mechanisms</u>
1) Plasma membrane calcium ATPase (PMCA)
Calcium pump with high affinity (0.2 μM)/low capacity for calcium. It is calmodulin-dependent.

2) Na^+/Ca^{2+} exchanger (NCX)
Na^+/Ca^{2+} antiporter on the plasma membrane with low affinity (1 μM)/high capacity. It can also reverse the transport direction. For instance, during depolarization, the increase of Na^+ inside the cell drives the NCX to pump Na^+ out of the cell and Ca^{2+} into the cell.

MECHANISMS OF Ca^{2+} OVERLOAD TOXICITY

A) Alteration of mitochondrial ATP synthesis
The excessive Ca^{2+} transport into mitochondria dissipates the mitochondrial membrane potential ⇒ inhibition of ATP synthase activity.

B) Increased ATP consumption due to the activation of pumps and transporters in the attempt to sequester/extrude Ca^{2+}.

C) A large increase of Ca^{2+} in mitochondria triggers the opening of the mitochondrial permeability transition pore (mPTP) with further dissipation of membrane potential and release of cytocrome C and apoptotic inducing factor (AIF) that can activate apoptosis.

D) Free radical production
- Ca^{2+} increases the activity of dehydrogenases (e.g. pyruvate DH ⇒ increased production of NADH ⇒ increased flux of electrons along the ETC + reduced ATP synthase activity ⇒ increased production of superoxide radical.

- Conversion of XDH into XO ⇒ increased production of superoxide radical.

- Excessive NO production by costitutive NOS ⇒ NO + O_2 ⇒ $ONOO^-$ ⇒ NO_2 CO_3 (OH)

E) Excessive activation of proteases (calpain) and degradation of proteins

F) Excessive activation of phospholipases (e.g. A2, D)

G) Excessive/altered activation of endonucleases (e.g. elevated calcium levels lock topoisomerase II in a form that is able to cleave but not to religate DNA)

H) Cytoskeleton alteration
High calcium concentrations can cause the dissociation of actin microfilaments.

AGENTS CAUSING ELEVATION OF CALCIUM

A) Receptor agonists (e.g. glutamate, kainate, domoate, capsaicin)

B) Agents altering VDCCs (e.g radicals)

C) Agents forming pores in the plasma membrane (e.g. maitotoxin, methylmercury)

D) Agents altering membrane permeability (e.g. detergents, phospolipases, lipid peroxidation inducers)

E) IP3 receptor activators (e.g. lindane)

F) Agents inducing release from mitochondria (e.g. fatty acid hydroperoxides, NO, $ONOO^-$, menadione)

G) Agents inhibiting Ca^{2+} extrusion (e.g. CCl4, benzene derivates, chloroform, cadmium)

H) Agents impairing ATP synthesis

3) Oxidative/nitrosative stress with alterations/fragmentation of lipids, proteins and DNA