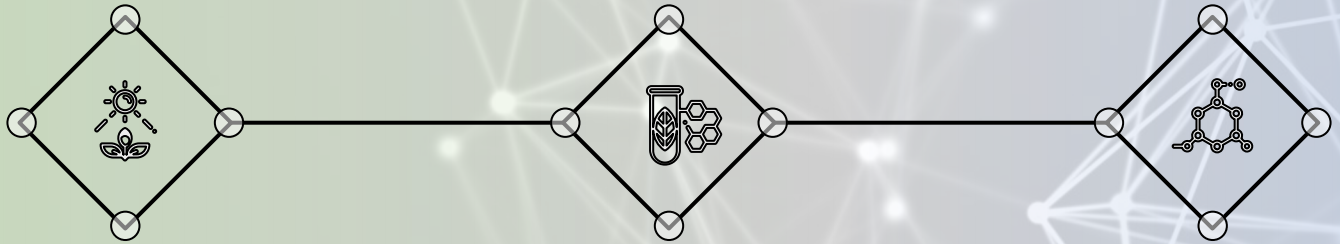
CINÉTICA E REGULAÇÃO ENZIMÁTICA | ENZYMOLOGY IN ECOTOXICOLOGY

BERNARDO DUARTE

MARE - Centro de Ciências do Mar e do Ambiente & Faculdade de Ciências da Universidade de Lisboa



ECOLOGY

Ecology is a branch of biology concerning **interactions among organisms and their biophysical environment**, which includes both biotic and abiotic components.

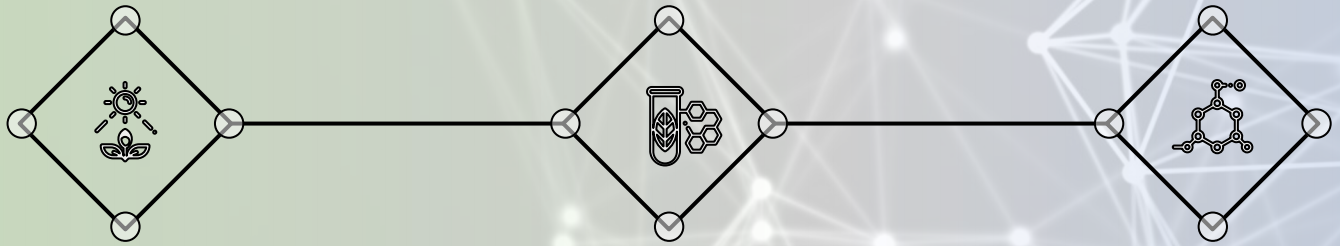
ECOTOXICOLOGY

The study of the **effects of toxic chemicals on biological organisms**, especially at the **population, community, ecosystem, and biosphere levels**. Ecotoxicology is a multidisciplinary field, which integrates toxicology and ecology.

In Ecotoxicology the concentration of the test substance in the target organisms should reflect the environmentally relevant or expected concentrations.

TOXICOLOGY

Toxicology is a scientific discipline, overlapping with biology, chemistry, pharmacology, and medicine, that involves the **study of the adverse effects of chemical substances on living organisms** and the practice of diagnosing and treating exposures to toxins and toxicants.



BIOINDICATOR

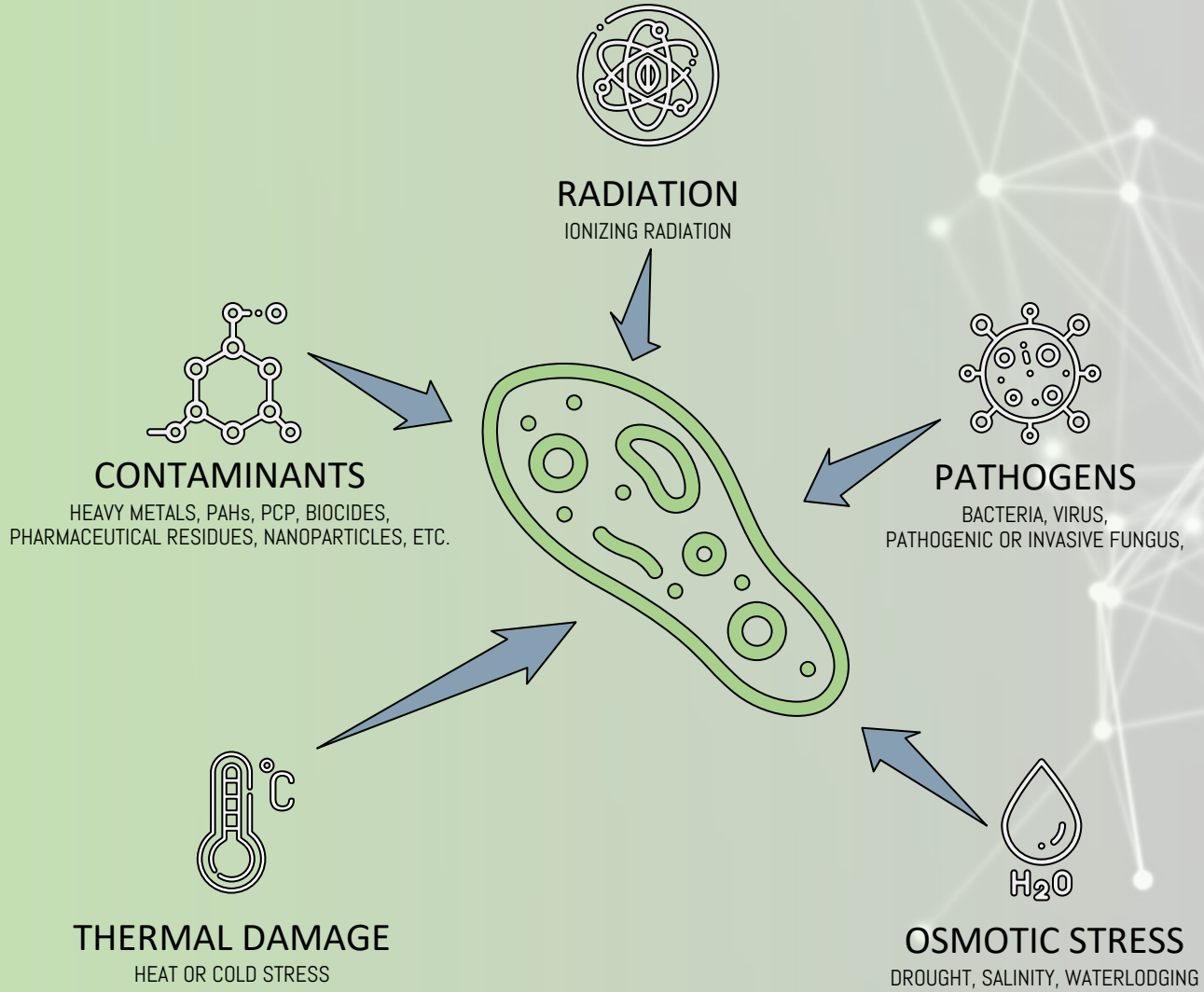
Organisms that express specific symptoms or responses that indicate environmental changes. Produce **QUALITATIVE** information regarding these changes (better, worse than a previous or reference condition).

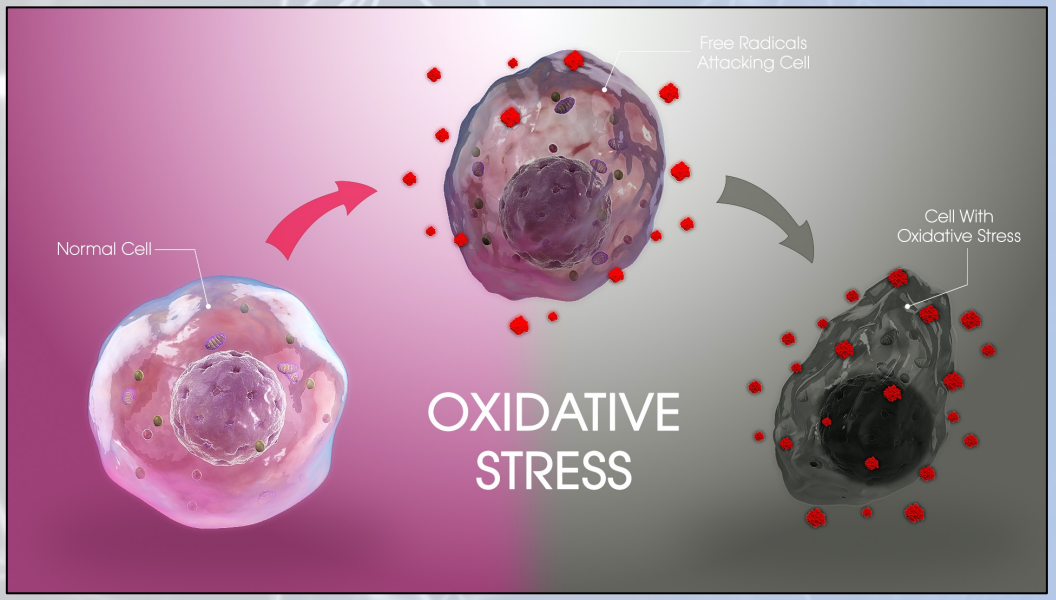
BIOMONITOR

Organisms or populations which the distribution is studied over time and space and compared to a model where the deviations to the expected behaviour are evaluated. Produce **QUANTITATIVE** information regarding the environmental changes.

BIOMARKER

A trait or molecular entity that can be measured experimentally and indicate the occurrence of a certain function (normal or pathological) of a certain organisms towards a specific stressor. Ideally these biomarkers should produce a dose related response towards the stressor applied.





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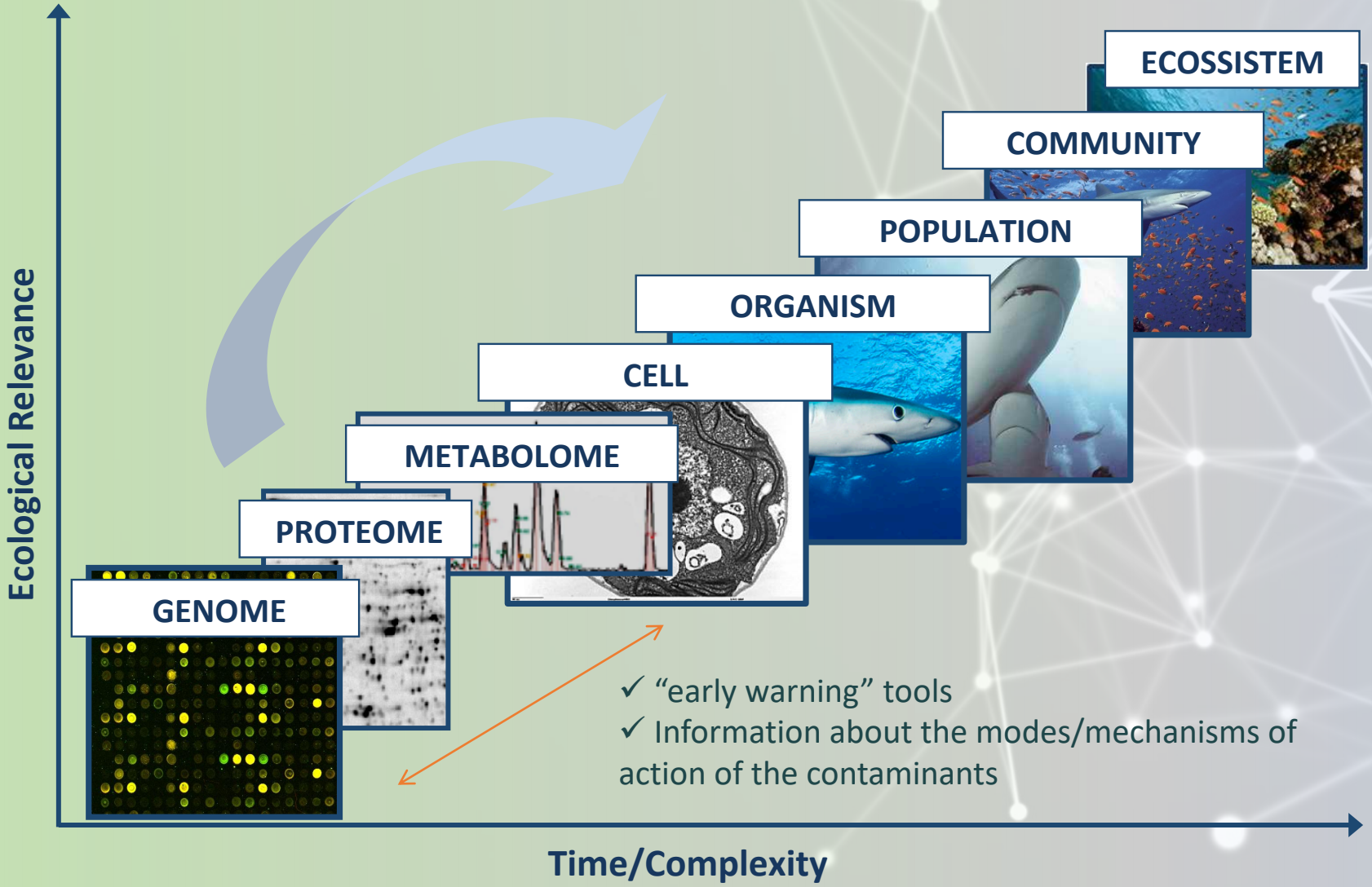


✓ "early warning" tools
✓ Information about the modes/mechanisms of action of the contaminants

Xenobiotic compound

Metabolic detoxification

✓ Attempt to restore homeostasis

→ Convert the xenobiotic into a less toxic/reactive molecule

→ Convert the xenobiotic into a more water-soluble (polar) molecule to be more easily excreted by the cell

Phase I (modification)

Phase II (conjugation)

Phase III (excretion)

Biotransformation

PHASE I: BIOTRANSFORMATION

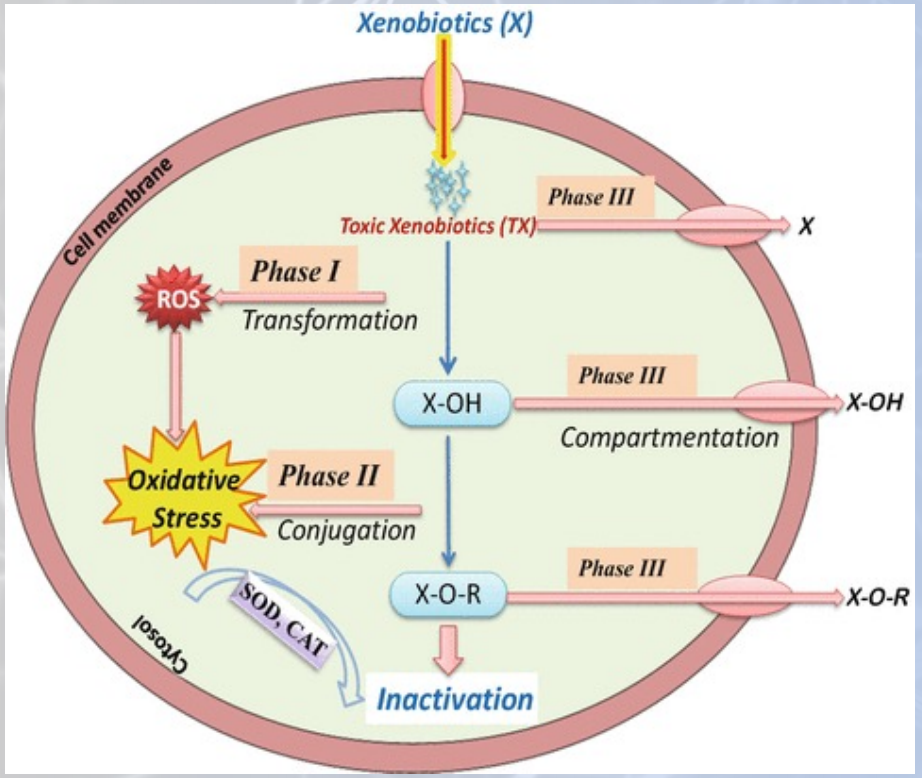
Phase I enzymes and mechanisms transform xenobiotics into less harmful molecules, but that may have a ROS-generating potential (for e.g.: CYPA1 enzymes).

PHASE II: CONJUGATION

Phase II mechanisms are composed by enzymatic and non-enzymatic anti-oxidant mechanisms that work in conjugation to quench the ROS generated directly by the xenobiotic or by the Phase I biotransformed xenobiotic.

PHASE III: EXCRETION

Phase III mechanisms are based in membrane proteins that can excrete directly or throughout vesicle compartments the transformed and/or inactivated xenobiotic to the extracellular environment.



Xenobiotic compound

…in ecotoxicology

Xenobiotic compound

Metabolic detoxification

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Phase I (modification)
Phase II (conjugation)
Phase III (excretion)

Biotransformation

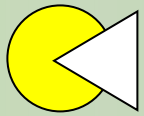
Xenobiotic compound

↓

Phase I biotransformation

↓

Reactive metabolite



- ✓ Adition of a functional group (OH, COOH... – more polar)
- ✓ Reactions include oxidation, reduction, hydrolysis

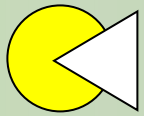
Xenobiotic compound

↓

Phase I biotransformation

↓ CYP450

Reactive metabolite



✓ Adition of a functional group (OH, COOH... – more polar)

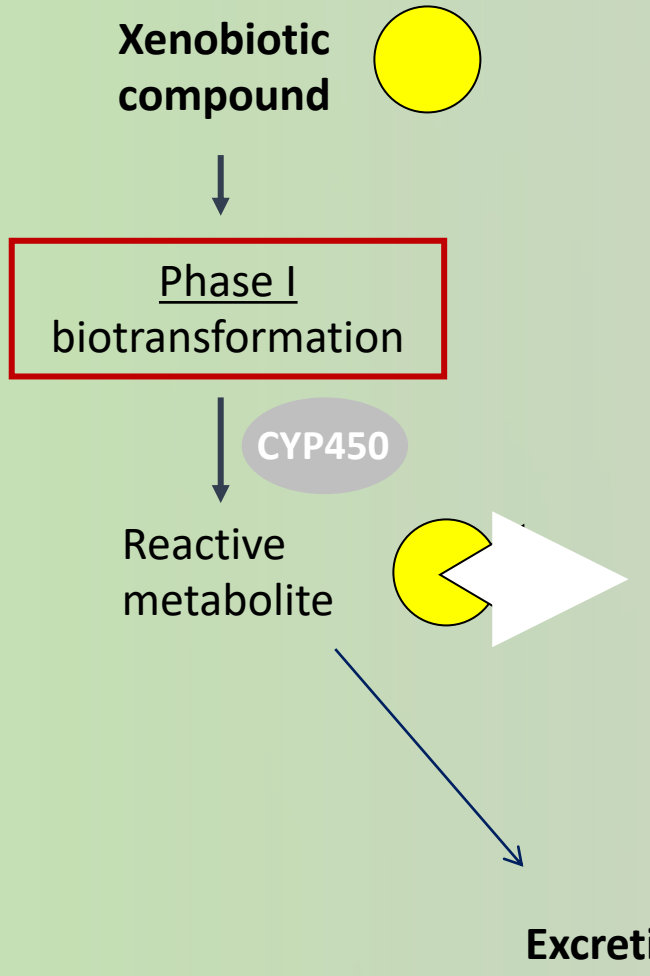
✓ Reactions include oxidation, reduction, hydrolysis

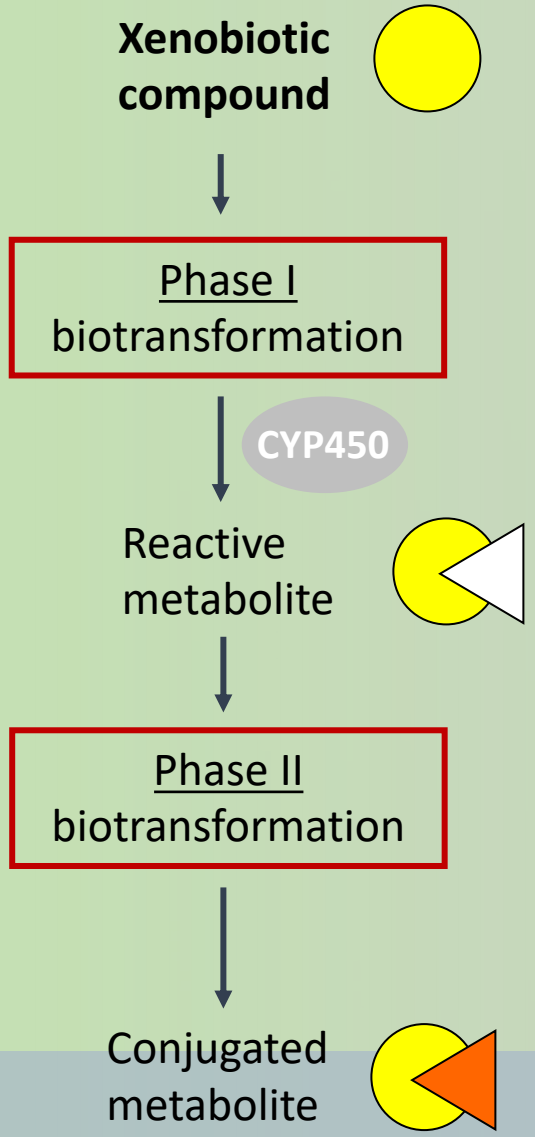
✓ These reactions usually involve a common enzyme system known as Cytochrome P450 family

-Hemoproteins identified in all kingdoms of life

- Oxidation of organic substances
  - xenobiotics
  - steroidal hormones
  - lipids

* Alcohol dehydrogenase (ADH)


Xenobiotic compound
Phase I biotransformation
CYP450
Reactive metabolite
Excretion

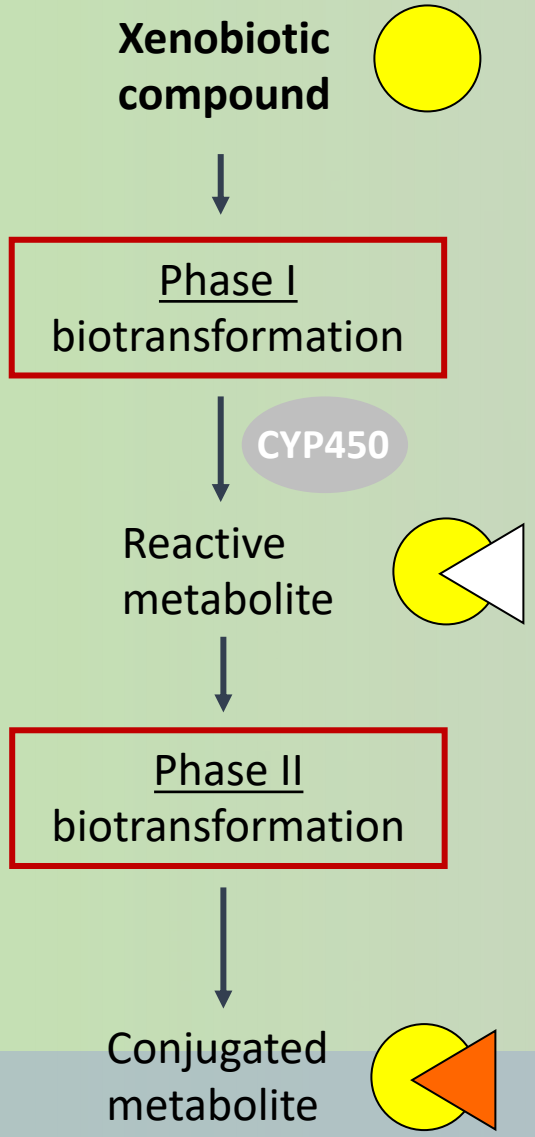


✓ Activated xenobiotic metabolites are conjugated with charged species - hydrophilic groups:

- glutathione (GSH)
- sulfate
- glycine
- glucuronic acid

✓ Conjugation reactions occur in carboxyl (-COOH), hydroxyl (-OH), amino (NH2), and sulfhydryl (-SH) groups of the xenobiotic metabolite – depending on the Phase I

✓ Catalyzed by a large group of broad-specificity transferases



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Glutathione S-transferases (GST)

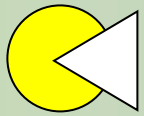
Xenobiotic compound

↓

Phase I biotransformation

↓ CYP450

Reactive metabolite



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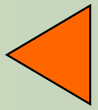
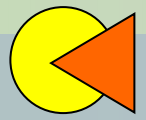
Phase II biotransformation

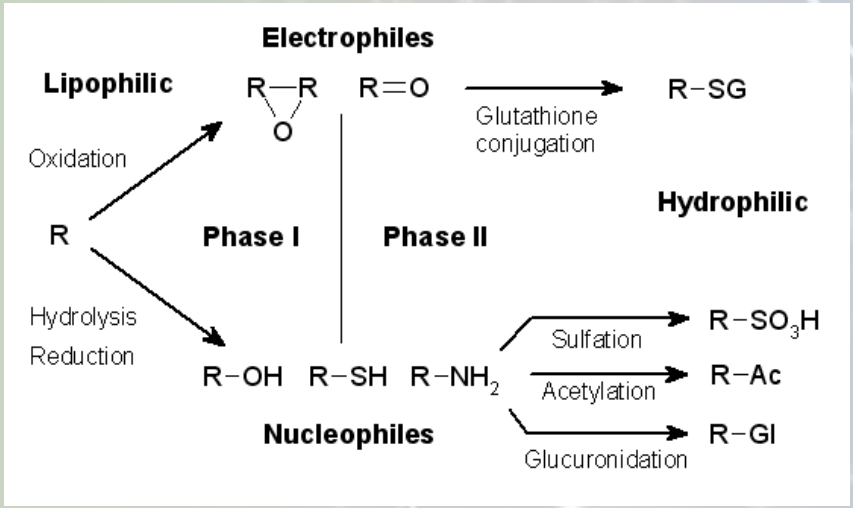
↓ GST + GSH



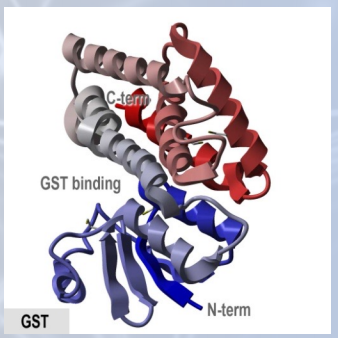
Conjugated metabolite



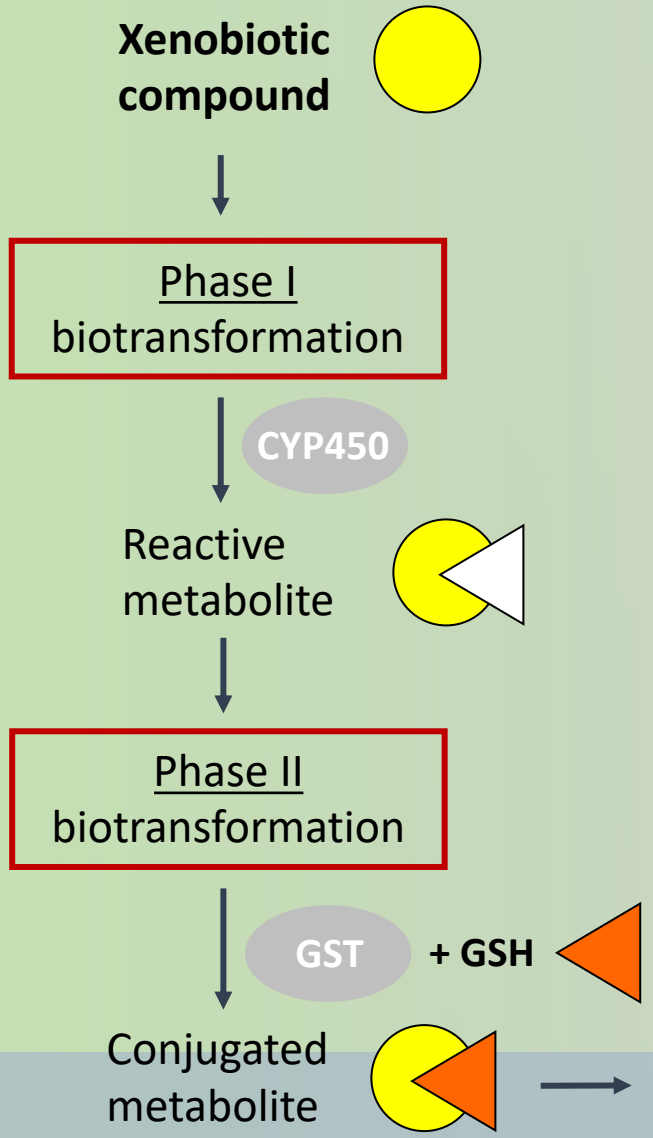
- ✓ Cytosolic, mitochondrial and microsomal
- ✓ Conjugation of electrophilic compounds with GSH



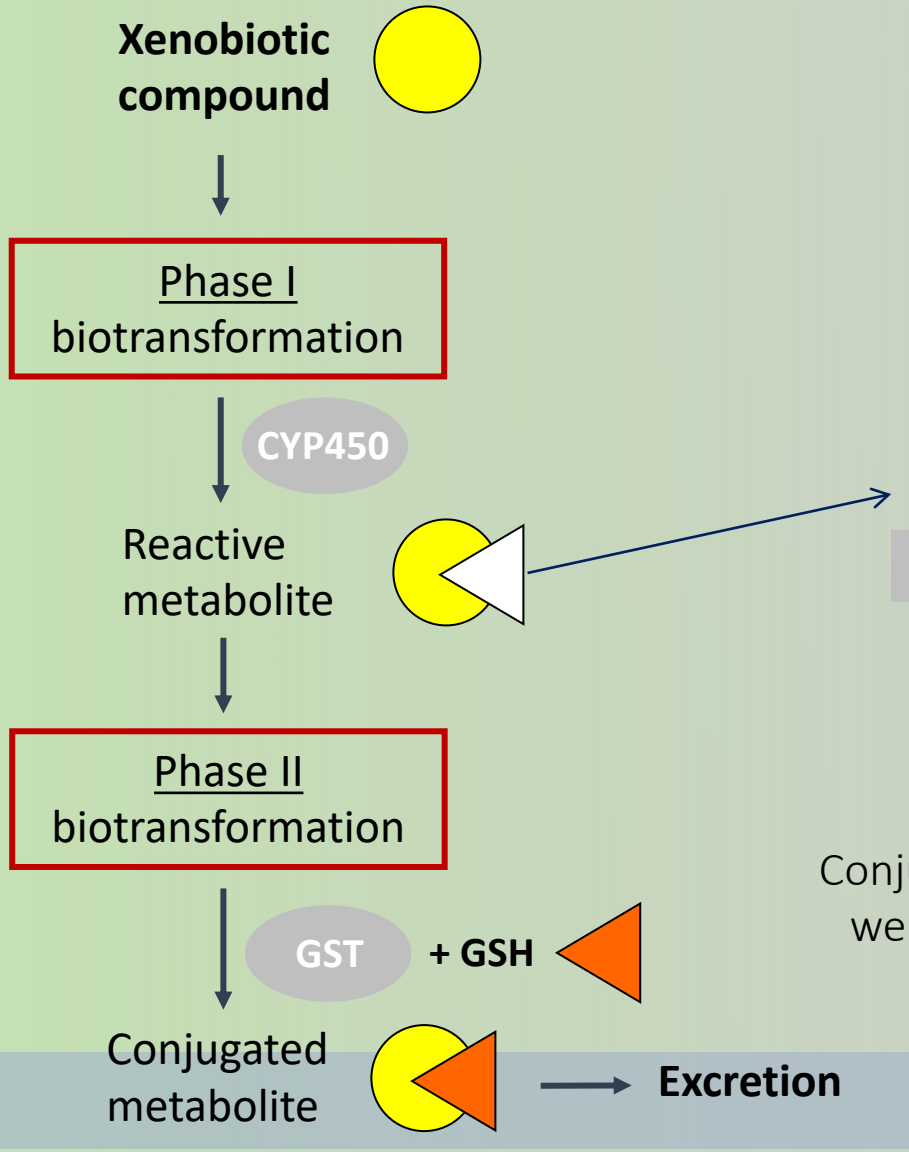
- ✓ May also bind directly to xenobiotics and function as transport proteins – “ligandins”



Glutathione S-transferases (GST)



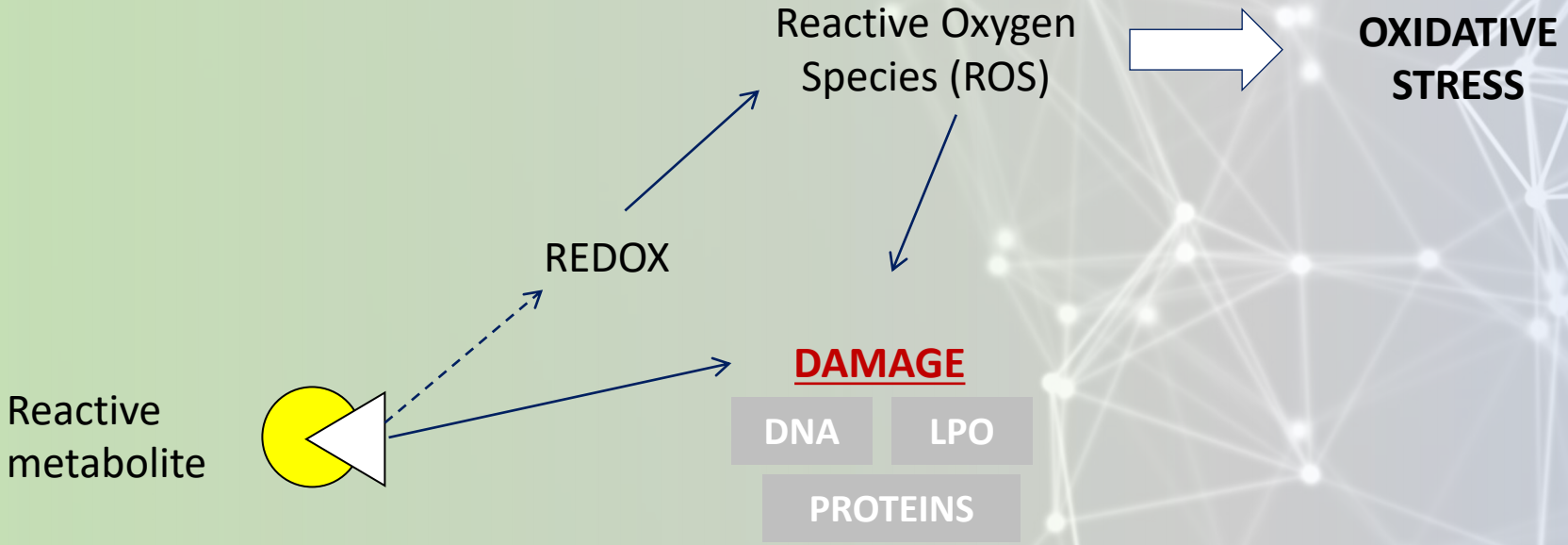
Conjugated metabolites = increased molecular weight and less active than their substrates

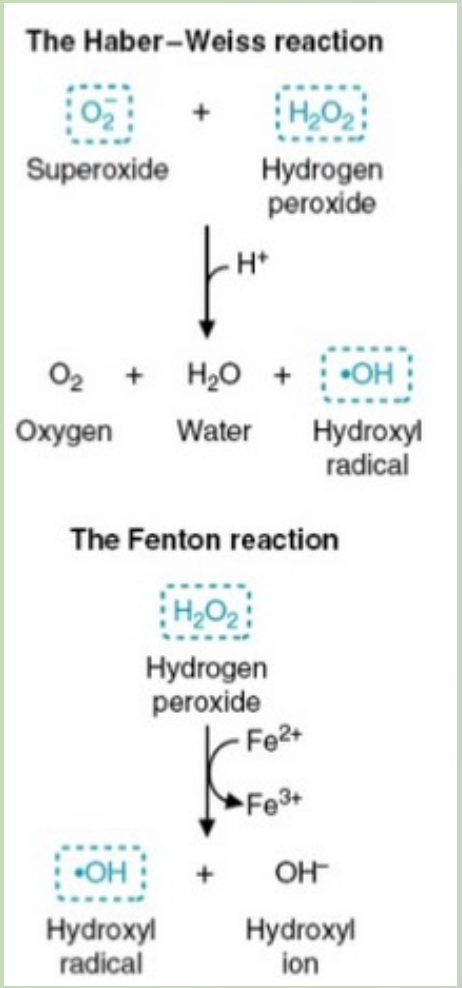


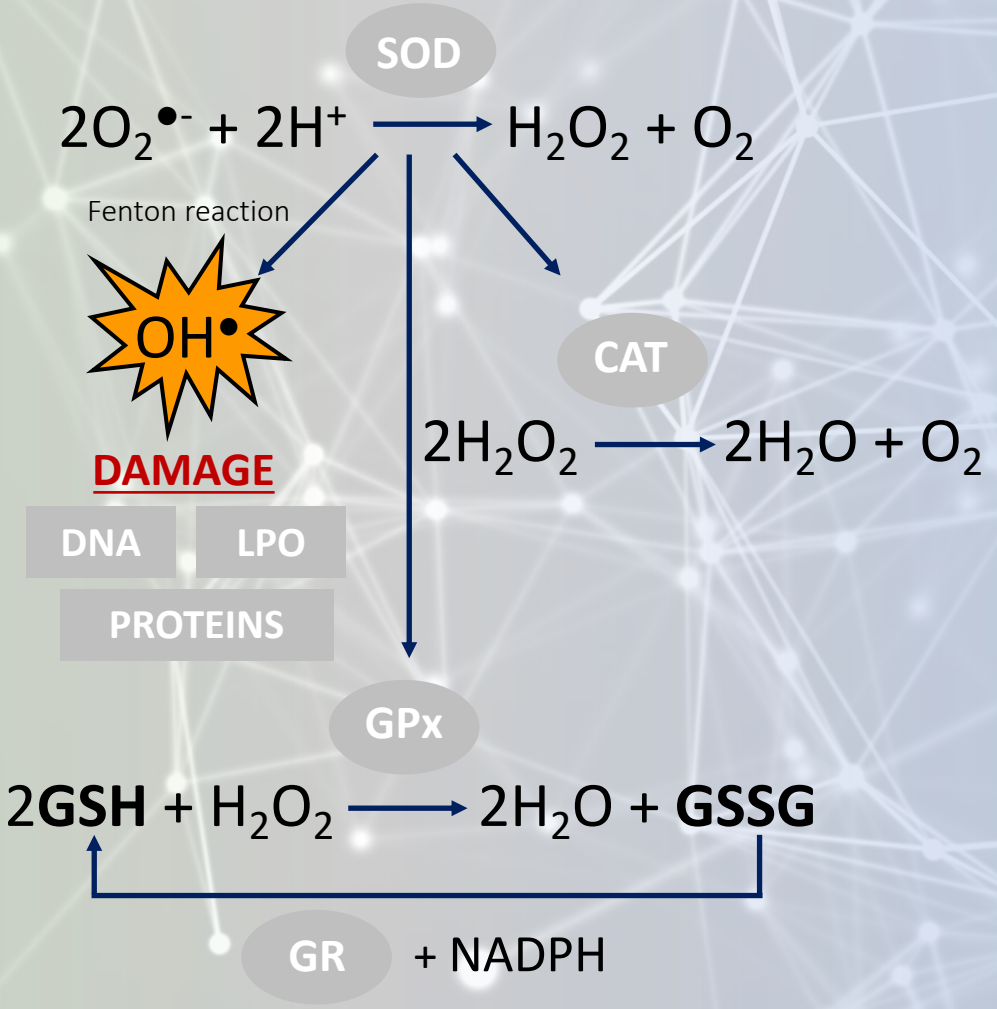
DAMAGE

DNA | LPO | PROTEINS

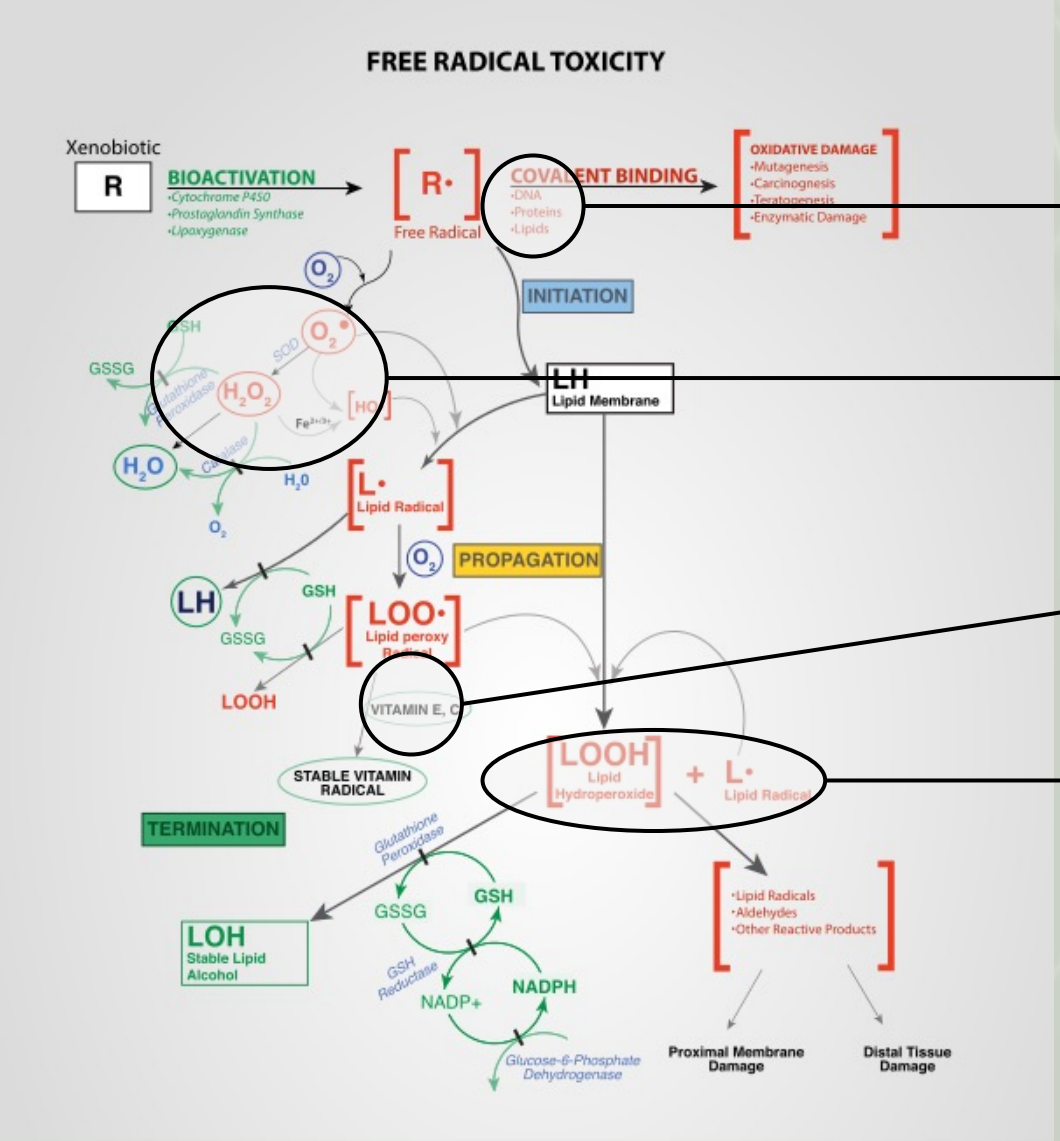
Conjugated metabolites = increased molecular weight and less active than their substrates


Reactive Oxygen Species (ROS)
OXIDATIVE STRESS
REDOX
DAMAGE
DNA
LPO
PROTEINS
Reactive metabolite





Adapted from Howcroft et al.,Environ. Int., 35, 318-324, 2009



DNA AND PROTEIN DAMAGE

ROS covalent binding to DNA leads to double strand disruption.
ROS interaction with Proteins induces protein oxidation/carboxylation.

ENZYMATIC DEFENSE SYSTEM

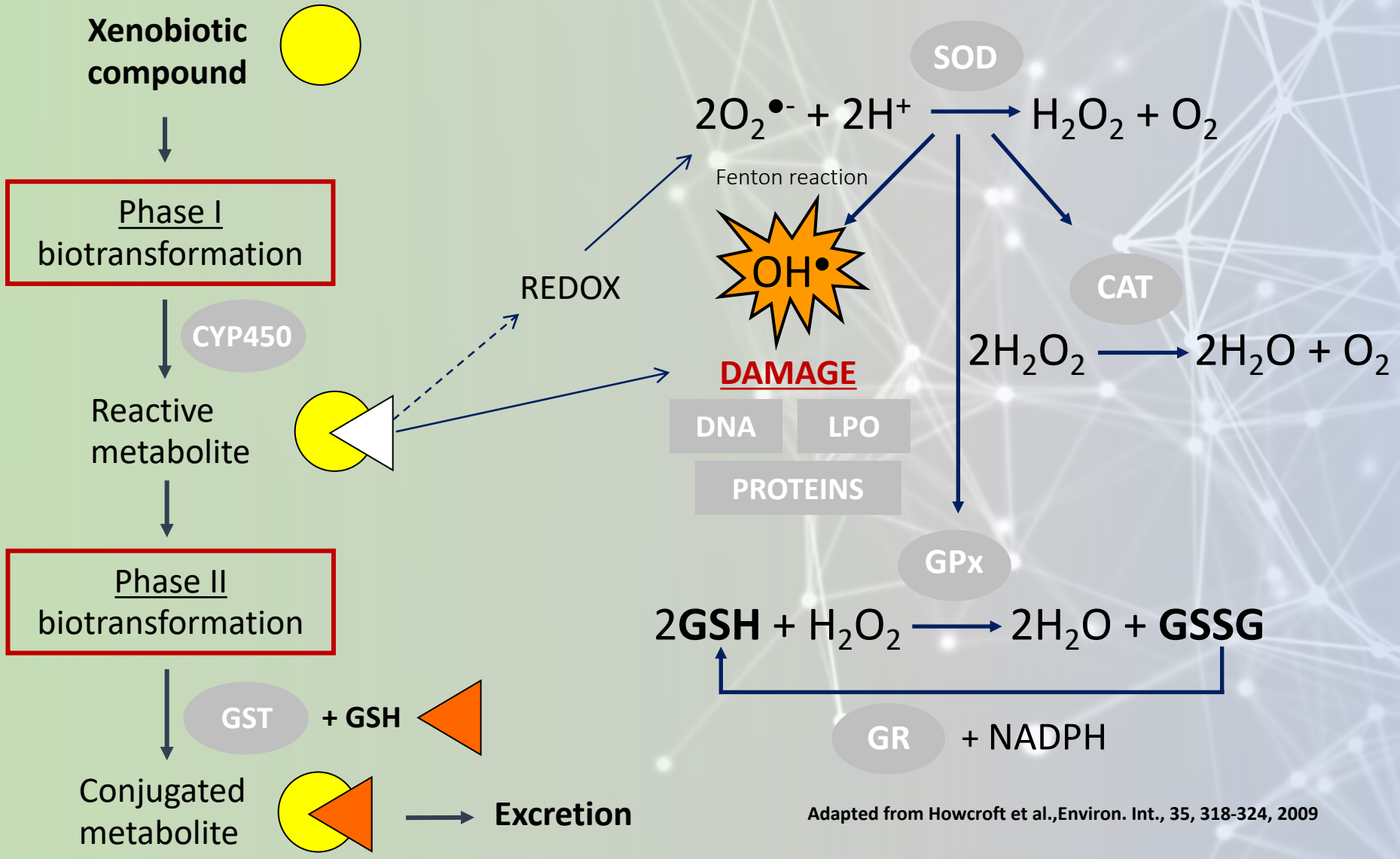
Superoxide dismutase isoforms and several peroxidases decompose ROS into harmless substances.

NON-ENZYMATIC ANTIOXIDANT

Vitamins, phenolics, flavonoids, thiol molecules and other anti-oxidant are able to quench directly ROS molecules into stable harmless radicals.

MEMBRANE DAMAGE

ROS interaction membrane fatty acids induces the formation of lipid hydroperoxides and lipid radicals inducing membrane disruption.



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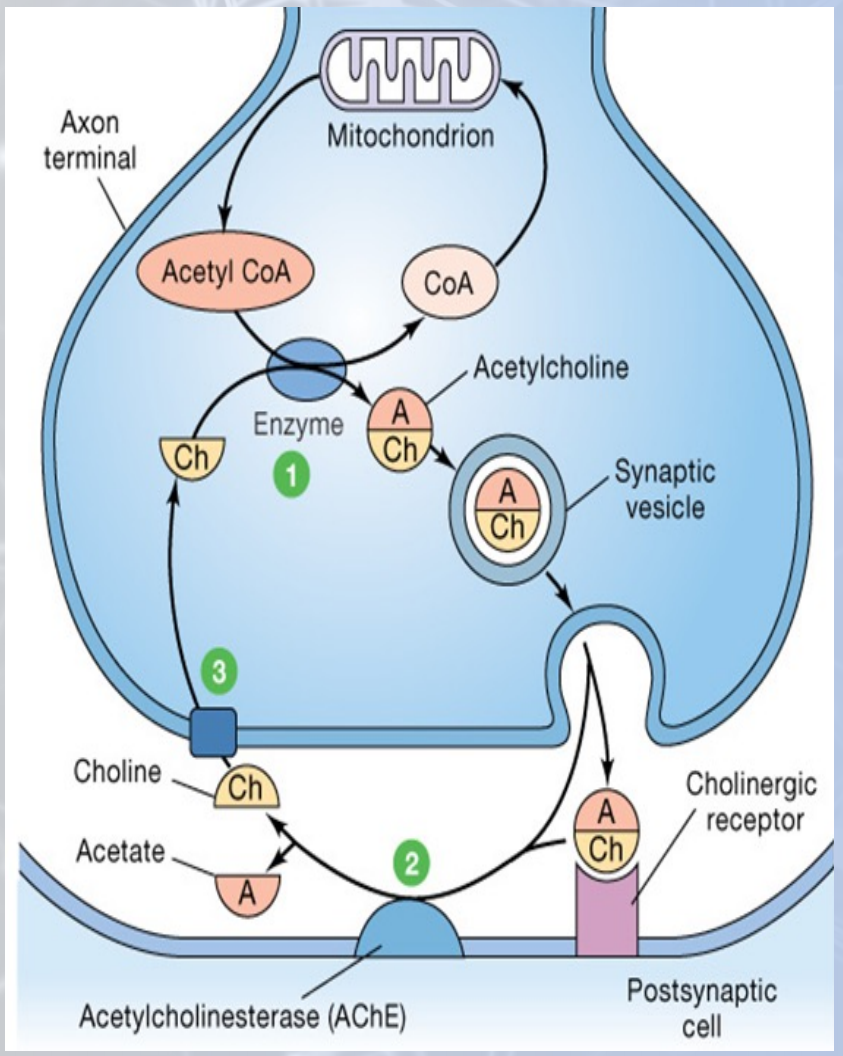
Role

- Hydrolysis and deactivation of acetylcholine
- Prevents acetylcholine reactivating receptor
- AChE plays an important role in neurotransmission in both vertebrates and invertebrates, being responsible for the hydrolysis of acetylcholine into choline and acetic acid at the cholinergic synapses and neuromuscular junctions.
- ChEs are a family of enzymes that includes acetylcholinesterase (AChE) or true cholinesterase and pseudocholinesterases (PsChE).



Acetylcholinesterase enzyme

Effect of inhibition

- Inhibitor blocks acetylcholinesterase
- Ach is unable to bind
- Ach returns to receptor and reactivates it
- Enzyme inhibitor has the same effect as a cholinergic agonist

Alzheimer's disease (AD), also known simply as Alzheimer's, is a **neurodegenerative disease** characterized by **progressive cognitive deterioration** together with declining activities of daily living and neuropsychiatric symptoms or behavioral changes. It is the most common type of dementia.

The most striking early symptom is **loss of short term memory (amnesia)**, which usually manifests as minor forgetfulness that becomes steadily more pronounced with illness progression, with relative preservation of older memories. As the disorder progresses, **cognitive (intellectual) impairment extends to the domains of language (aphasia), skilled movements (apraxia), recognition (agnosia), and those functions (such as decision-making and planning)** closely related to the frontal and temporal lobes of the brain as they become disconnected from the limbic system, reflecting extension of the underlying pathological process. These changes make up the essential human qualities, and thus AD is sometimes described as a disease where the victims suffer the loss of qualities that define human existence.

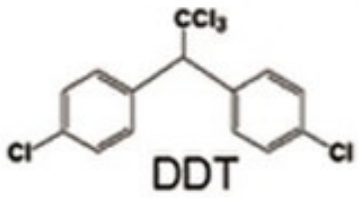
This **pathological process consists principally of neuronal loss or atrophy**, principally in the temporoparietal cortex, but also in the frontal cortex, together with an inflammatory response.

Acetylcholinesterase inhibitors

Acetylcholinesterase (AChE) inhibition was thought to be important because there is a reduction in activity of the cholinergic neurons. AChE-inhibitors reduce the rate at which acetylcholine (ACh) is broken down and hence increase the concentration of ACh in the brain (combatting the loss of ACh caused by the death of the cholinergin neurons). Acetylcholinesterase-inhibitors seemed to modestly moderate symptoms but do not alter the course of the underlying dementing process

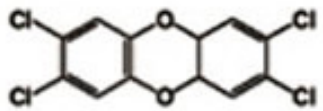
Emerging AChE disruptors

Organo-chlorinated pesticides



DDT

Dioxins and dioxin-like PCBs

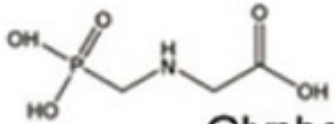


Dioxins

PAHs

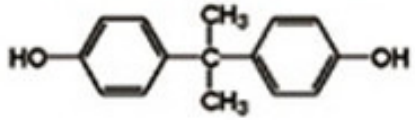
B(a)P

Herbicides



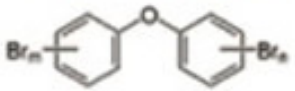
Glyphosate

Plasticizers



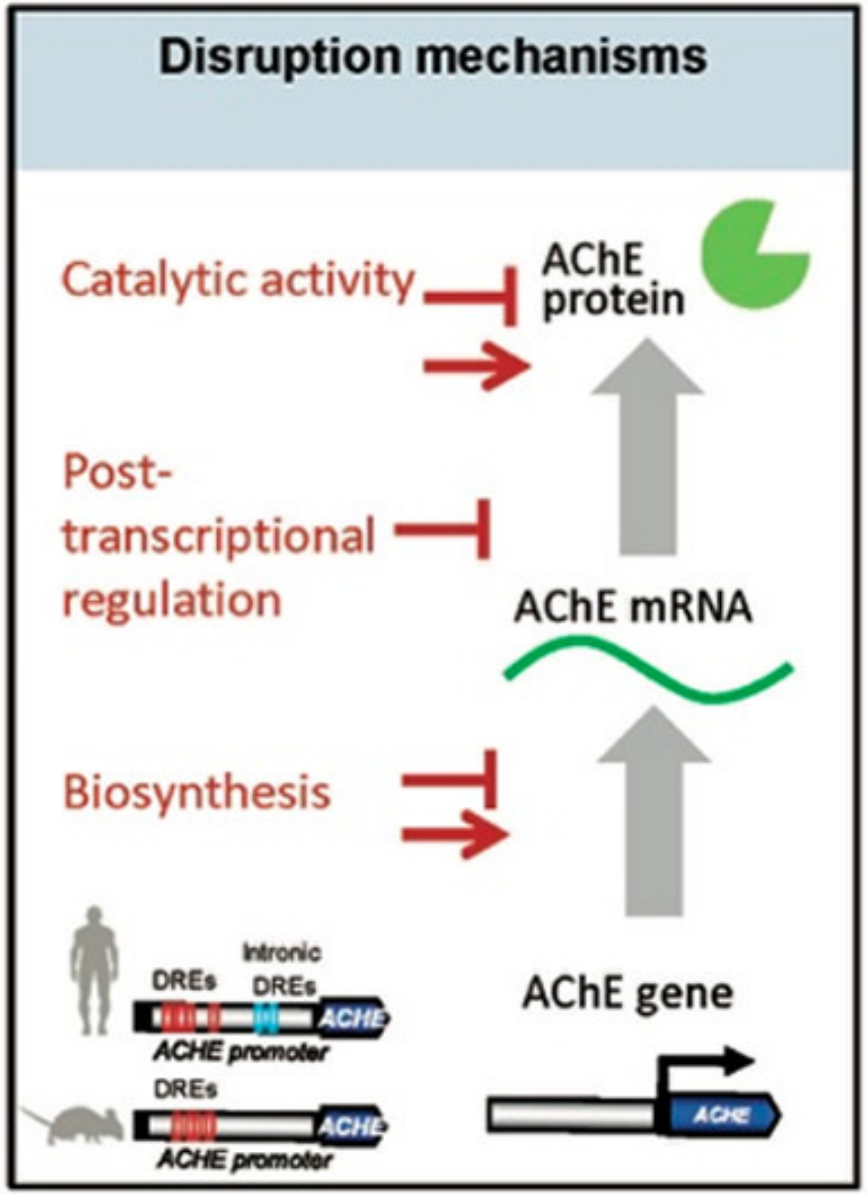
BPA

Flame retardants



PBDE

Disruption mechanisms



ACETYLCHOLINESTERASE INHIBITORS

Some of the most toxic pesticides are insecticides

Some of the most toxic insecticides are cholinesterase inhibitors

- Organophosphates (OP) and N-methyl – carbamates are the insecticidal cholinesterase inhibitors
- Cholinesterase screening may be necessary

Who to monitor (EPA directives)?

- Individuals who apply, load, or mix Class I or II Organophosphate pesticides or Organophosphates and N-methyl - Carbamates
- Working 30 or more hours within any 30-day period

What to measure (EPA directives)?

- Measure both acetylcholinesterase (red blood cell cholinesterase-AChE) and butyryl cholinesterase (plasma cholinesterase-PChE)
- Use the same laboratory and the same methodology for all testing so that results may be accurately compared. Repeat baselines yearly
- >20% decrease from baseline in AChE or PChE = Evaluate work practices
- >30% decrease in AchE or > 40% decrease in PChE

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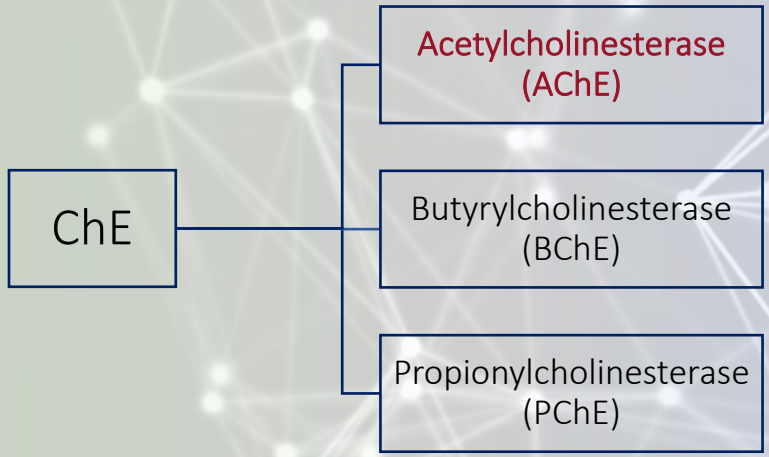
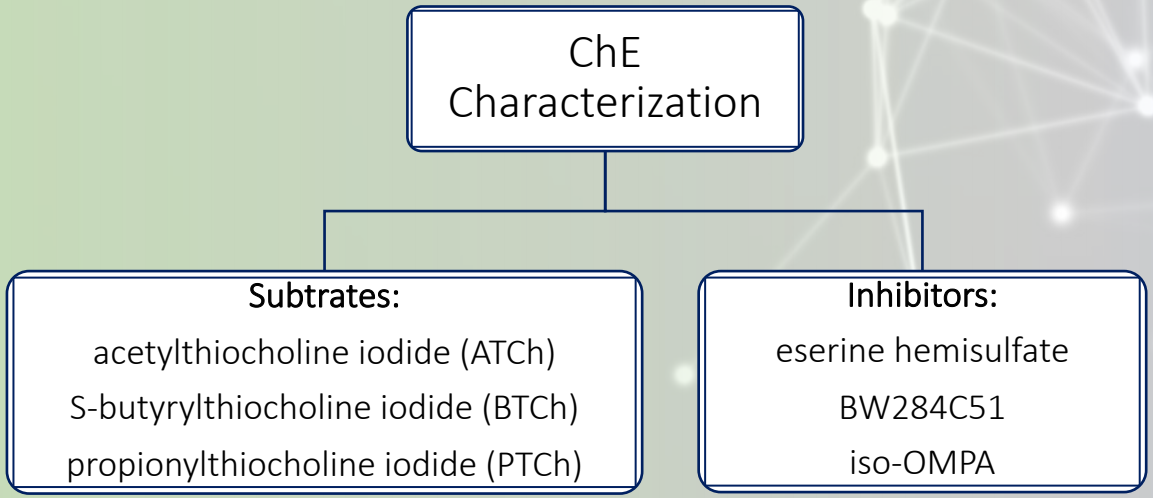
Acetylcholinesterase (AChE)

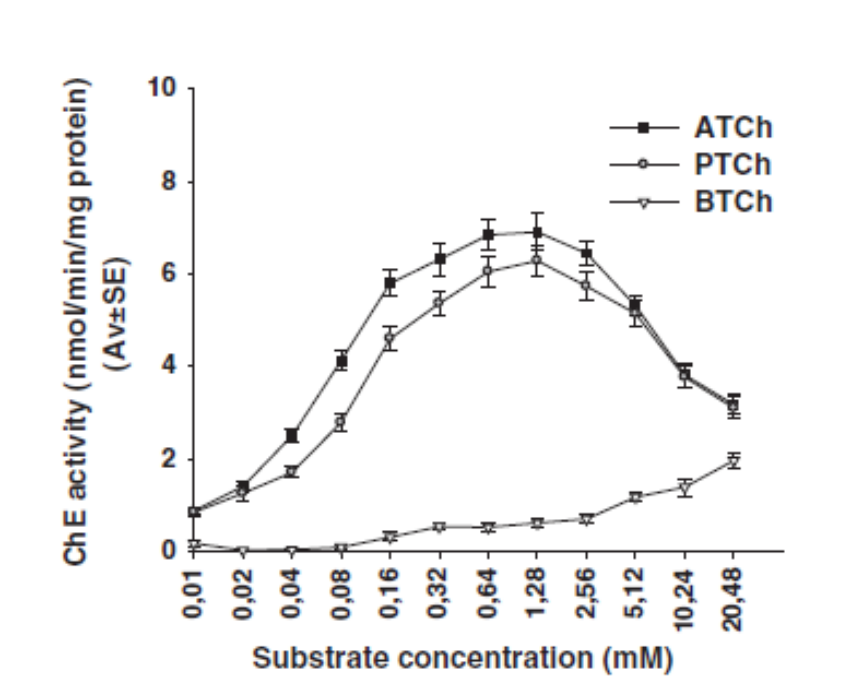
Cholinesterases (ChE) are a family of enzymes that play an essential role in neuronal and motor functions



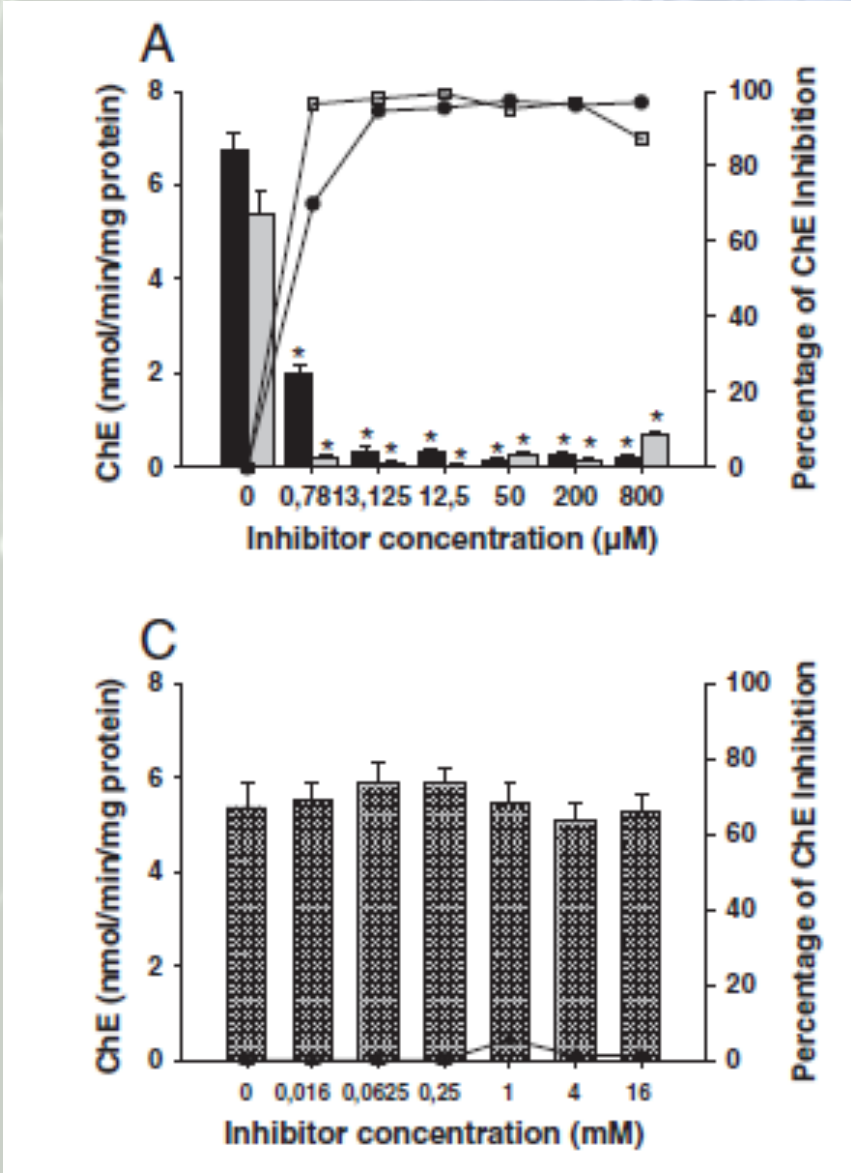


Use of selective inhibitors

- **Eserine** – ACETYLCHOLINESTERASE inhibitor (also known as Physostigmine, a highly toxic parasympathomimetic alkaloid, specifically, a reversible cholinesterase inhibitor).
- **BW284C51** - PROPIONYLTHIOCHOLINESTERASE inhibitor (also known as 1,5-bis (4-allyl-dimethylammoniumphenyl) pentan-3-one dibromide)
- **iso-OMPA** – BUTYRYLTHIOCHOLINESTERASE inhibitor (also known as tetra (monoisopropyl) pyrophosphor-tetramide



Typical AChE



Consider one organism sampled in two different sites with different degrees of pollution.

First Step: We need to characterize which Cholinesterase is prevalent in the organism that will be used as biomonitor?

- **Eserine** – ACETYLCHOLINESTERASE inhibitor (also known as Physostigmine, a highly toxic parasympathomimetic alkaloid, specifically, a reversible cholinesterase inhibitor).
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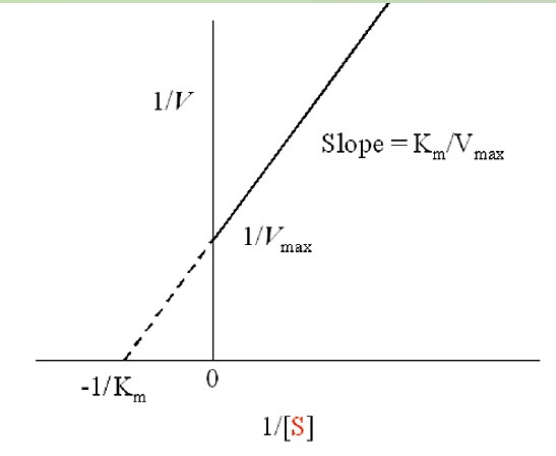
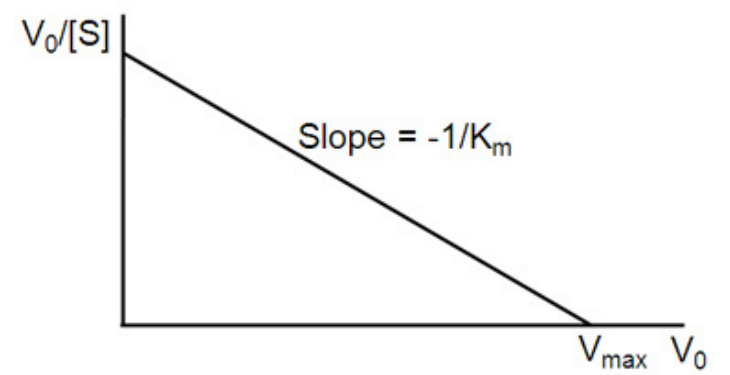
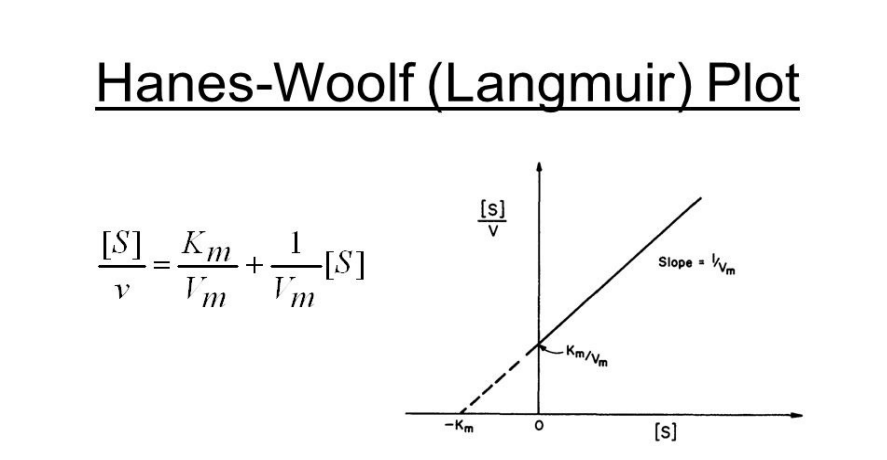
Consider one organism sampled in two different sites with different degrees of pollution.

Second Step: Considering the prevalent Cholinesterase which site is more contaminated, having as basis the Km and Vmax of this enzyme in the individuals from both sites?

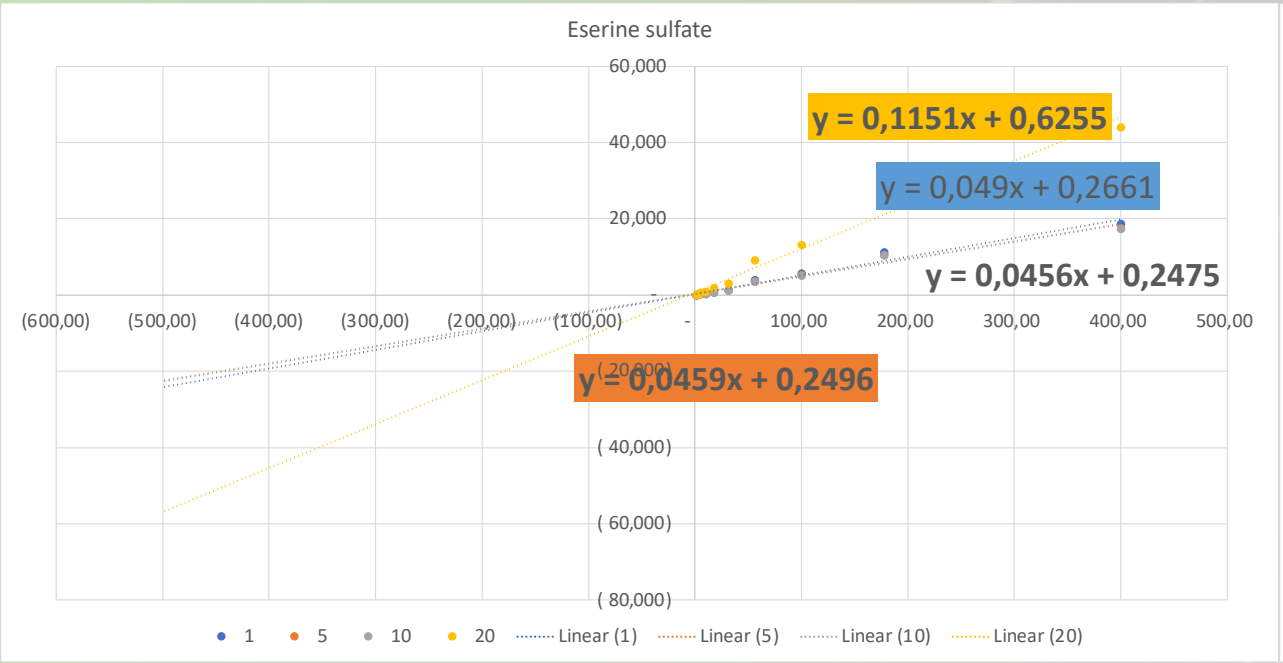
- **Lineweaver-Burke**
- **Eadie–Hofstee**
- **Hanes-Woolf**

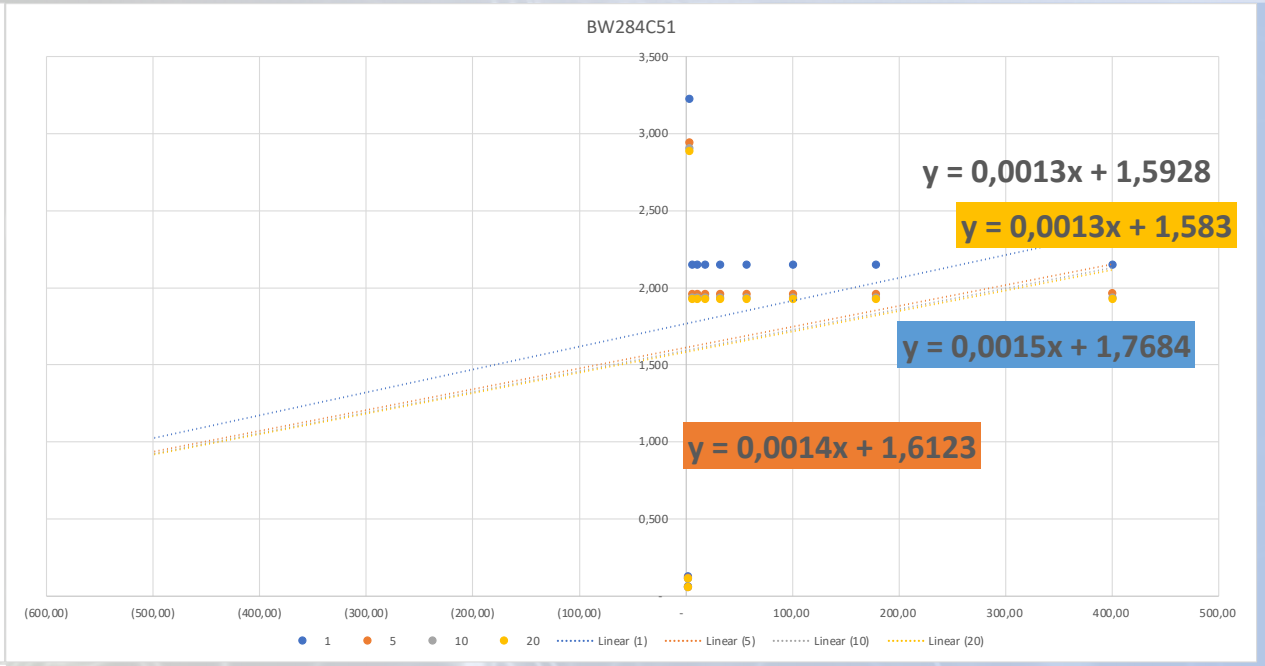


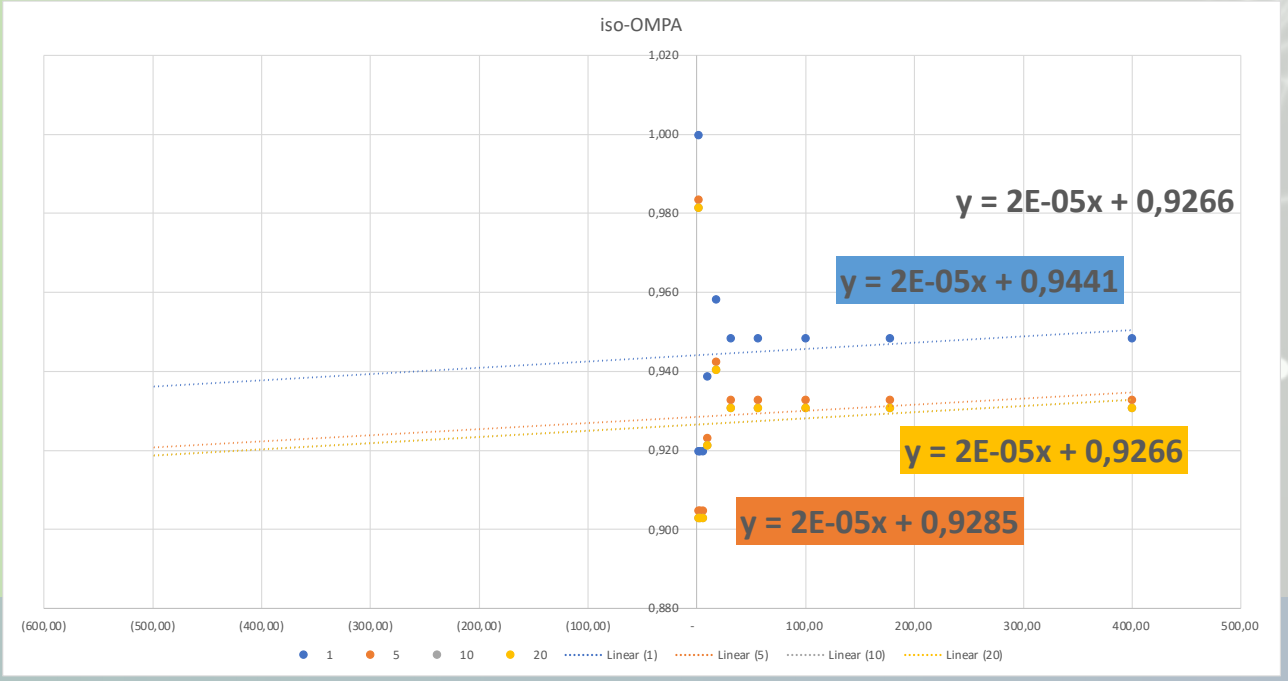




EXERCISES

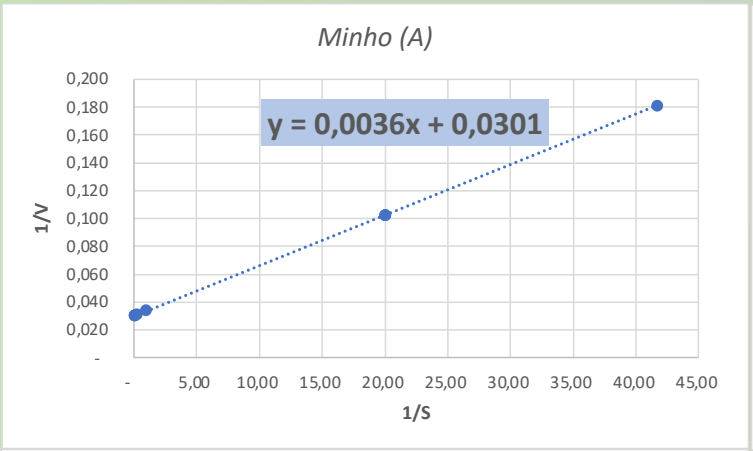


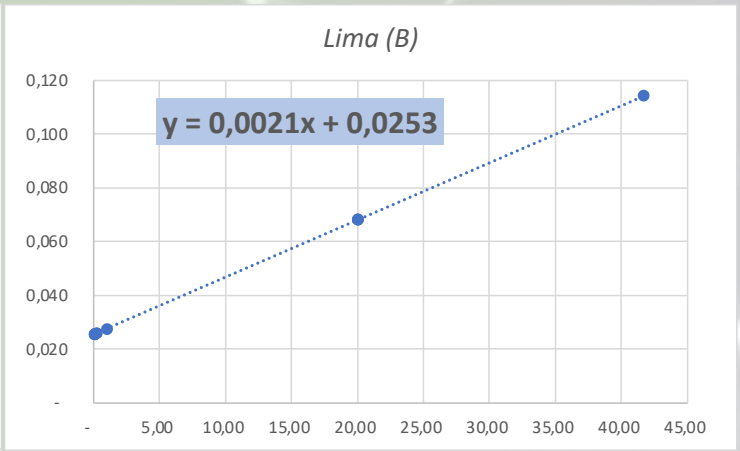


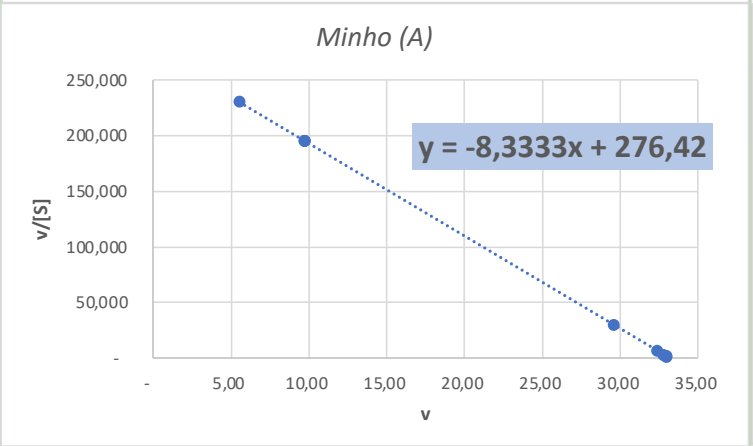


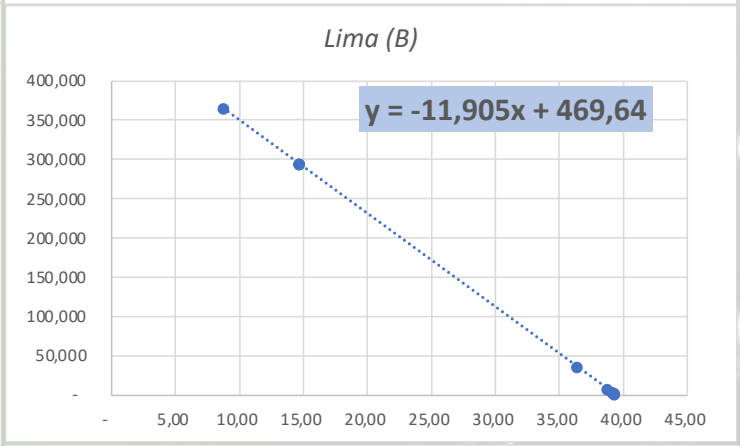
(Eserine sulfate)				
[S]	**1**	**5**	**10**	**20**
m	0,049	0,0459	0,0456	0,1151
b	0,2661	0,2496	0,2475	0,6255
Ki	5,43	5,44	5,43	5,43
(BW284C51)				
[S]	**1**	**5**	**10**	**20**
m	0,0015	0,0014	0,0013	0,0013
b	1,7684	1,6123	1,5928	1,583
Ki	1 178,93	1 151,64	1 225,23	1 217,69
(iso-OMPA)				
[S]	**1**	**5**	**10**	**20**
m	0,00002	0,00002	0,00002	0,00002
b	0,9441	0,9285	0,9266	0,9266
Ki	47 205,00	46 425,00	46 330,00	46 330,00

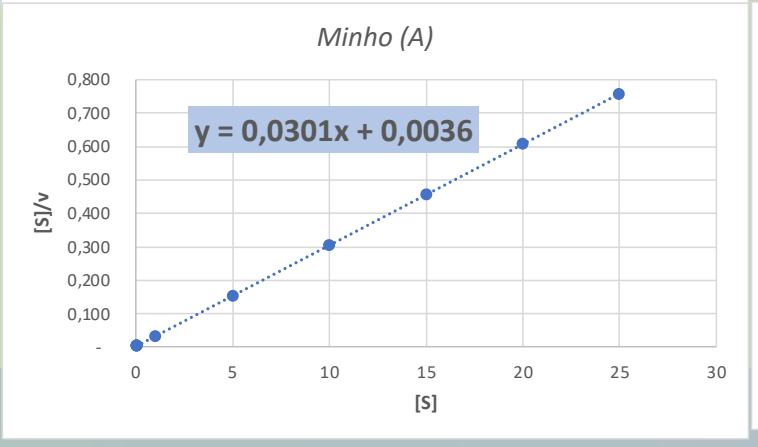
BERNARDO DUARTE (BADUARTE@FC.UL.PT) CRE 2022-23

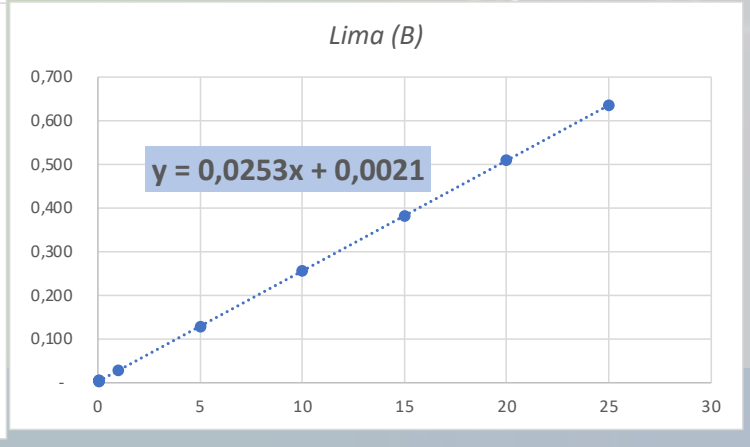












	Minho (A)	Lima (B)
Lineweaver–Burk		
Km (Acetylthiocholine)	0,12	0,083
Vmax (Acetylthiocholine)	33,22	39,53
Eadie–Hofstee		
Km (Acetylthiocholine)	0,12	0,08
Vmax (Acetylthiocholine)	33,17	39,45
Hanes-Woolf		
Km (Acetylthiocholine)	0,12	0,08
Vmax (Acetylthiocholine)	33,22	39,53

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