Development of Regional Joint Master Program in Maritime Environmental Protection and Management
- MEP&M -

Main pollutants in the environment and some tools for their analysis (Part 2)

WP3. Capacity Building through staff training and equipment purchase .
Dev 3.4.2 KNOW-HOW TRANSFER TO TEACHING STAFF RELATED TO THE MEP&M

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20 December 2021

Virtual meeting via Google-meet application

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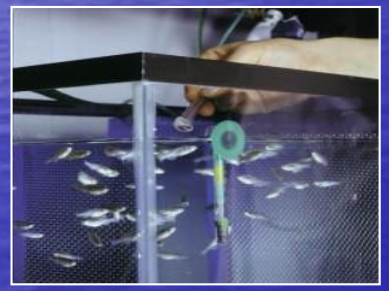
TOXICITY BIOASSAYS AND BIOMARKERS AS POLLUTION EFFECTS ASSESMENT TOOLS

TOXICITY BIOASSAYS

- To know **toxic effects / alterations** of pollutants on the organisms.
- To **establish water quality standards**, that is, define safety limits or acceptable concentrations of a pollutant.



Introduction to toxicity bioassays

Terminology

Toxicity

Adverse effects that pollutants cause in an organism

Exposure time

Time in which the organism is exposed to the solution under study

Acute toxicity

Lethal or other effect produced in a relatively short time, usually within 4 days for fish or macroinvertebrates and shorter periods (2 days) for smaller organisms

Chronic toxicity

Long-term effects that may be related to changes in the rate of feeding, growth, metabolism, reproduction, and even mutations and death

Introduction to toxicity bioassays

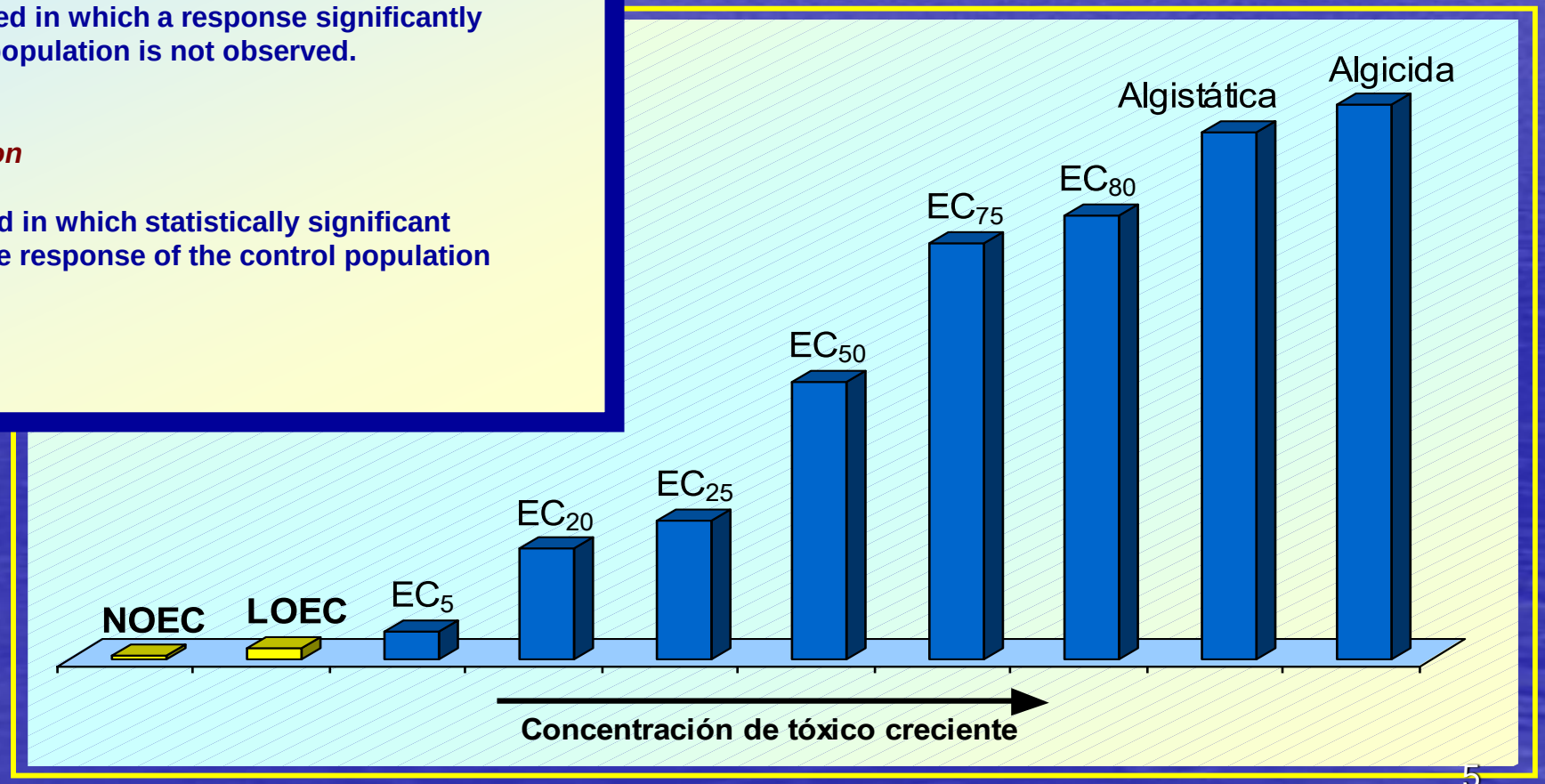
Terminology

NOEC, Non observed effect concentration

The highest concentration of pollutant tested in which a response significantly different from that obtained in the control population is not observed.

LOEC, Lowest observed effect concentration

The lowest concentration of pollutant tested in which statistically significant differences are observed with respect to the response of the control population



Introduction to toxicity bioassays

Terminology

ECx, Effective concentration

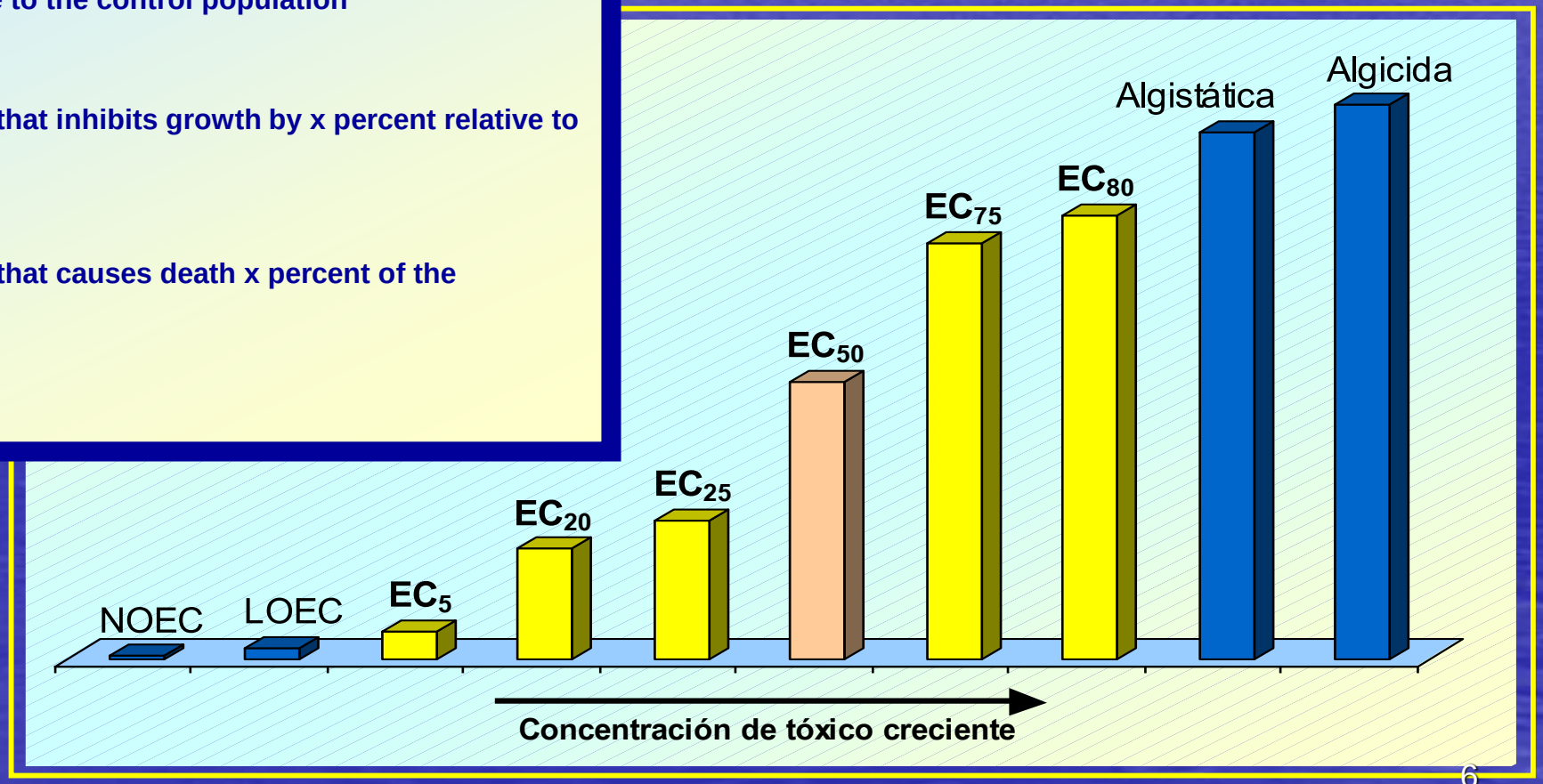
The estimated concentration of a pollutant that reduces the measurement parameter (e.g. growth, mortality) by x percent relative to the control population

ICx, Inhibition concentration

The estimated concentration of a pollutant that inhibits growth by x percent relative to the control population

LCx, Letal concentration

The estimated concentration of a pollutant that causes death x percent of the population relative to the control



Microalgae toxicity tests

Plant communities are very important for the functioning of aquatic ecosystems

Algae associated to plankton, that is, phytoplankton, form the basis of most food chains, produce oxygen, and play a key role in the nutrient cycle.

PHYTOTOXICITY tests are necessary to evaluate the impact of potential pollutants that can be introduced into the aquatic environment.

Introduction to toxicity bioassays

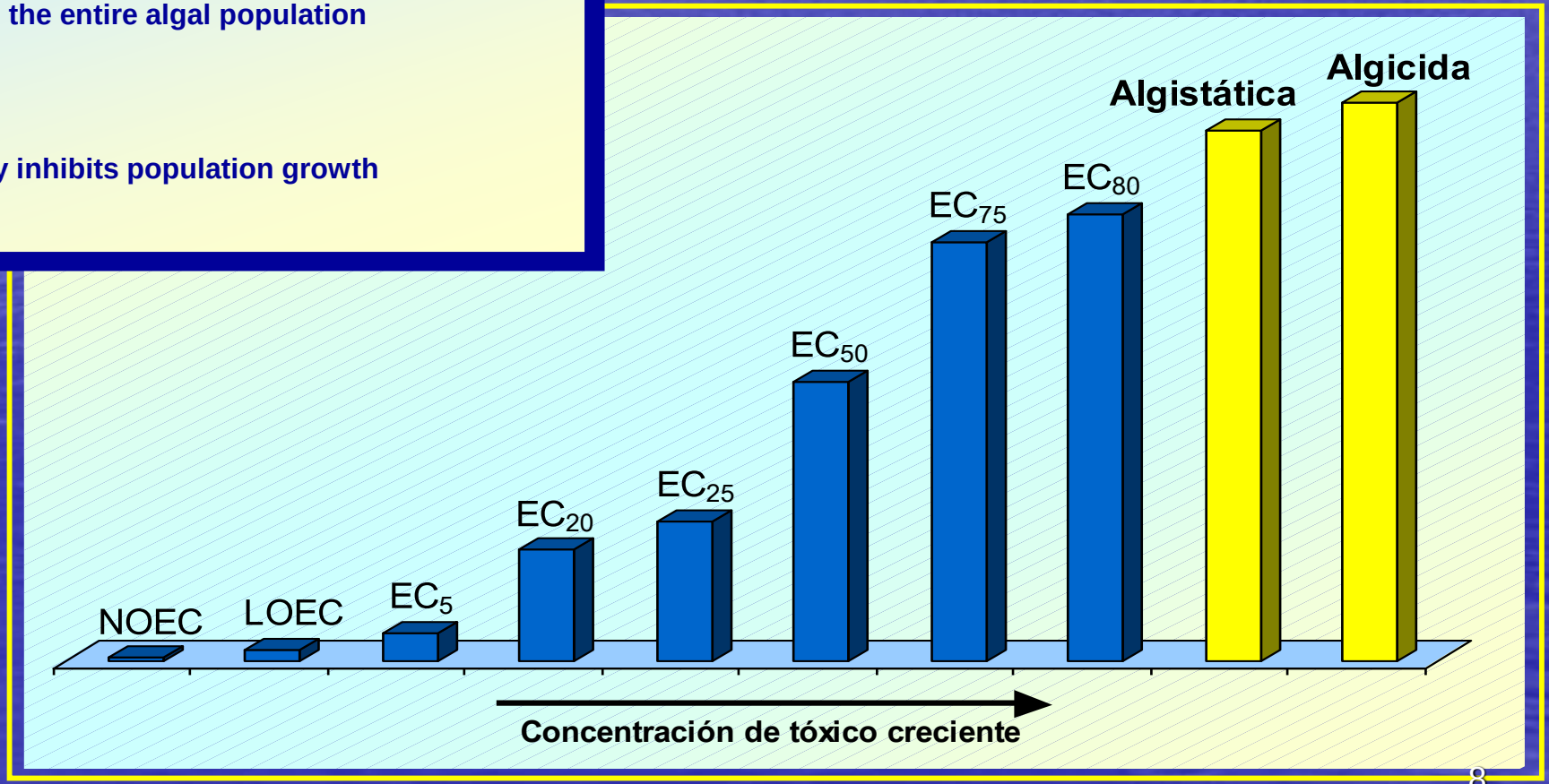
Terminology

Algaecide / algaecide concentration

Concentration of a pollutant that is lethal to the entire algal population

Algistatic / alguistatic concentration

Concentration of a pollutant that completely inhibits population growth

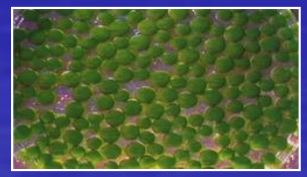


Microalgae toxicity tests

Testing method

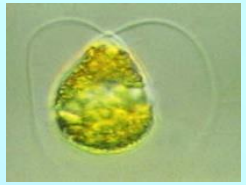
Most of the toxicity tests with algae are chronic tests since the effects are evaluated over several generations during the 3 to 4 days of exposure period.

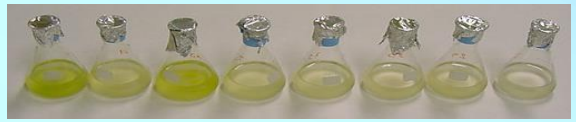
- ➔Static tests
- ➔72 or 96 hours exposure time
- ➔Nutrient enriched medium (N, P, Fe, vit., etc.)
- ➔Daily agitation for gas exchange
- ➔Controlled light and temperature conditions



PARAMETERS TO MEASURE TOXICITY

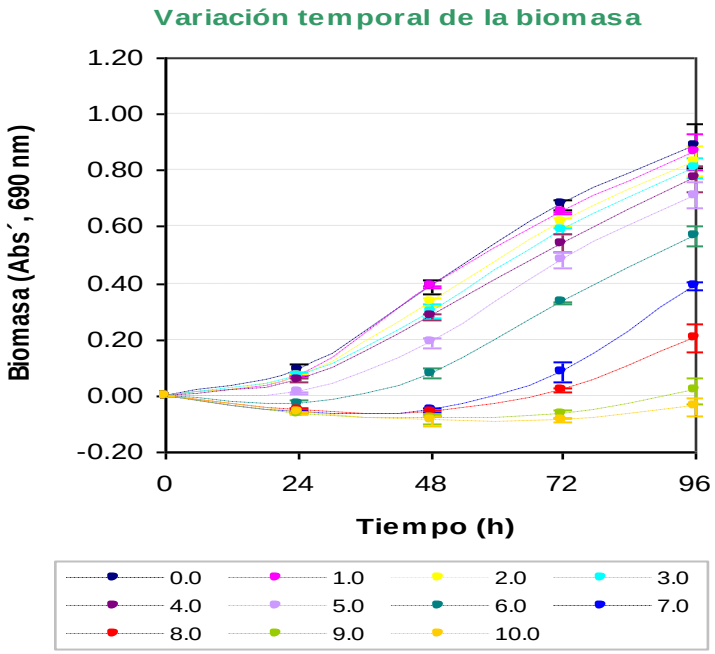
Microalgae: Dunaliella

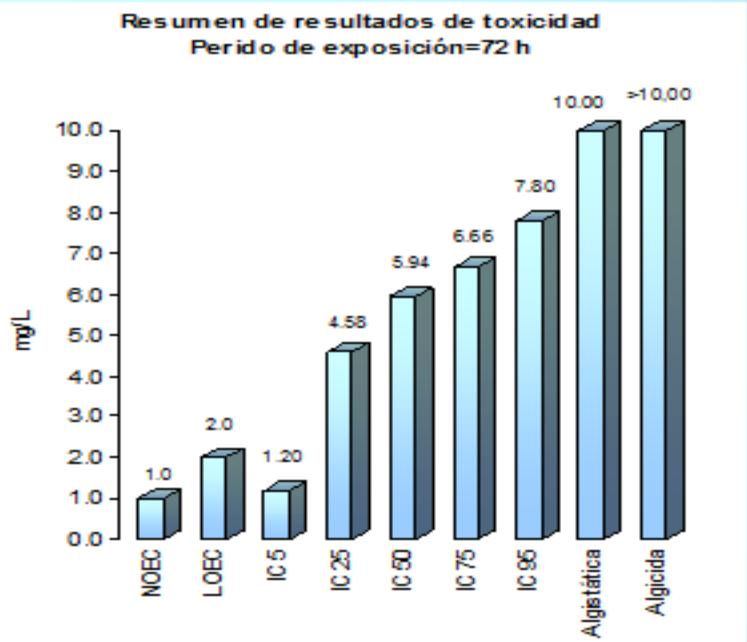


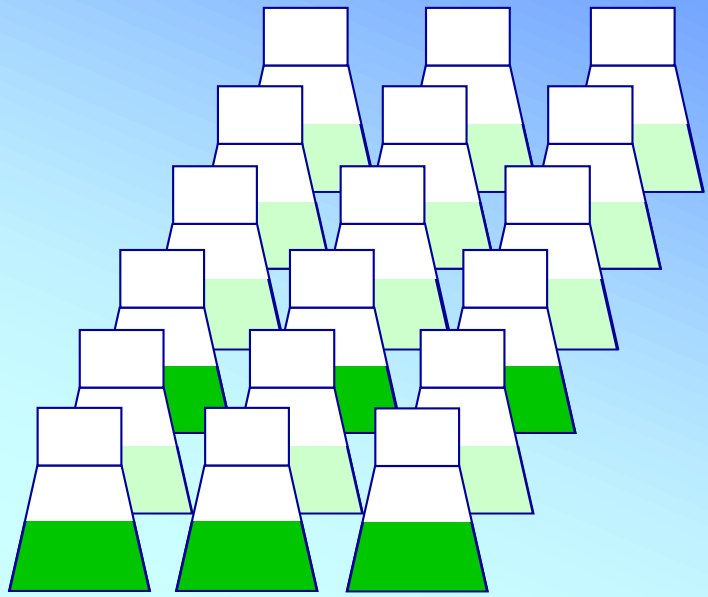


Dunaliella salina, Tóxico=LAS

NOEC
LOEC
Ic_x (IC_{50})







t= 72 o 96 horas
Biomasa final

Microcrustacean toxicity tests

Artemia is a crustacean suitable for the development of toxicity bioassays in seawater

Additional interest: its use in marine aquaculture

They develop resistance cysts, easily decapsulable in laboratory

Microcrustacean toxicity tests

Stage selection for toxicity tests

Vanhaecke et al. (1980) establishes three categories:

- Cysts
- Nauplius
- Adult

PARAMETERS TO MEASURE TOXICITY

Microcrutaceans: Artemia

Toxicity parameters in cysts:

- Percentage of hatched.

Toxicity parameters in nauplius:

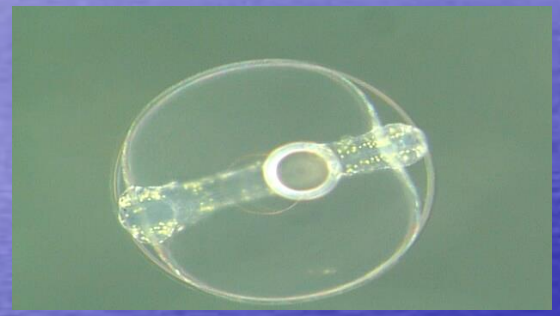
- Life-death criterion
- Immobilization of the organisms

Toxicity parameters in adults:

- Death of the organism
- Fecundity
- Reproductive capacity
- Bioaccumulation of toxins

Fish toxicity tests

- **EARLY DEVELOPMENT STAGES OF FISH**



24 H eggs of gilthead

72 H larvae of gilthead

- **JUVENILES OF FISH**

50 days juveniles of Senegal sole

GILTHEAD (*Sparus aurata*) EARLY DEVELOPMENTAL STAGES TOXICITY TEST

TYPE OF TEST

-Acute Interval Toxicity Assay with static character

-Exposure time: 48 hours (eggs)

96 hours (larvae)

-Number of replications: 3

TEST MATERIAL

-Two liter capacity borosilicate glass containers

-Dilution medium: Filtered seawater (0.45 µm)

-Aeration system

-Stereomicroscope

TEST PROCEDURE

50 organisms per container

Aeration (oxygen saturation)

Analysis of physico-chemical parameters of water

Count of living and dead organisms

Taking samples of water and organisms (morphological and histopathological changes)




TOXICITY TEST WITH SENEGAL SOLE JUVENILES

Solea senegalensis

TYPE OF TEST

-Acute Toxicity Assay with static character

-Exposure time: 96 hours

-Number of replications: 3

TEST MATERIAL

-Two liter capacity borosilicate glass containers

-Dilution medium: Filtered seawater (0.45 µm)

- Aeration system

-Stereomicroscope

TEST PROCEDURE

- 15 organisms per container
- Gentle aeration (oxygen saturation)
- Analysis of physico-chemical parameters of water
- Count of living and dead organisms
- Taking samples of water and organisms (histopathological changes)

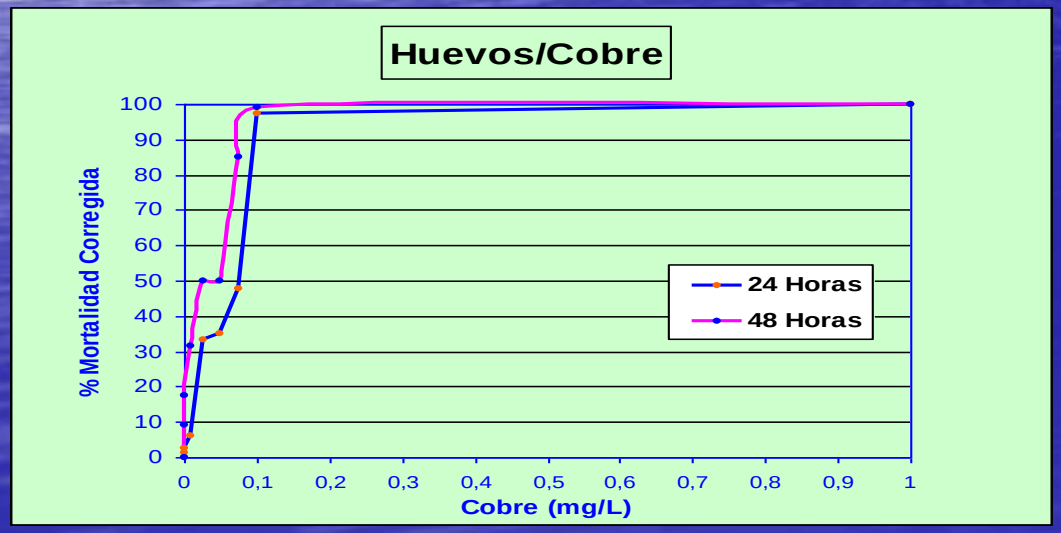
PARAMETERS TO MEASURE TOXICITY

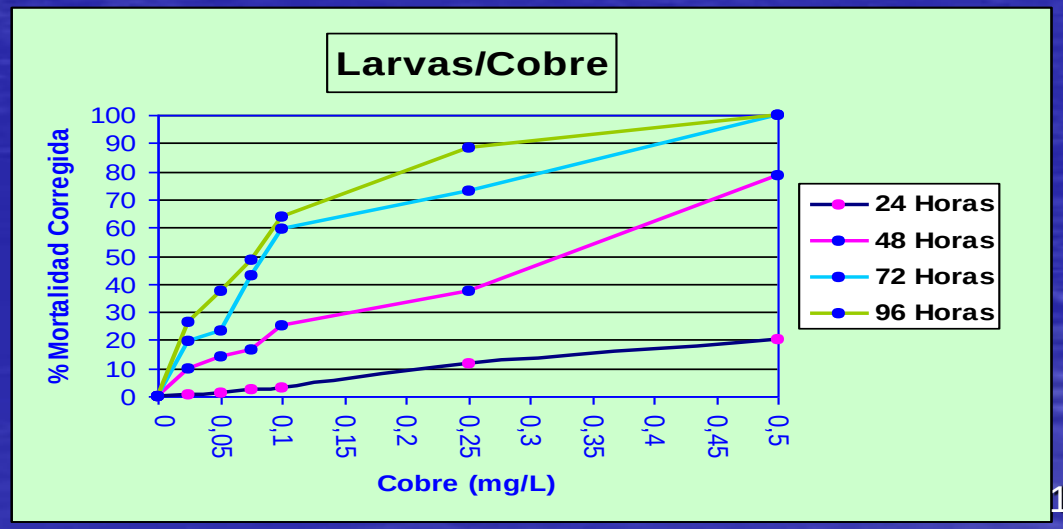
Calculation of Corrected Mortality

% Survival = (Nº Living organisms / Total Nº organisms) x 100

% Mortality = 100 -% Survival

$$\text{Corrected Mortality} = \frac{\text{\% Mortality -\% Control Mortality}}{\text{100 -\% Mortality Control}} \times 100$$





PARAMETERS TO MEASURE TOXICITY

LC_{50} ESTIMATION METHODS

Graphic method

Spearman-Karber method

Spearman-Karber Trimmer method

Probit method ...

- ✓ More formal procedure
- ✓ Statistical procedure
- ✓ LC50 and 95% confidence intervals

Description of the methods ...

http://www.epa.gov/OST/WET/

PROBIT program….

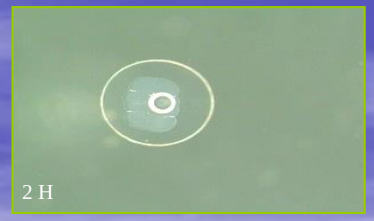
http://www.epa.gov/nerleerd/stat2.htm

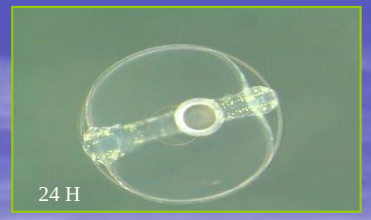
Estimation of CL_{50} in gilthead early life stages

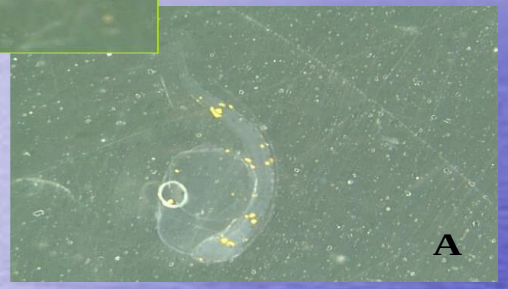
Probit Method

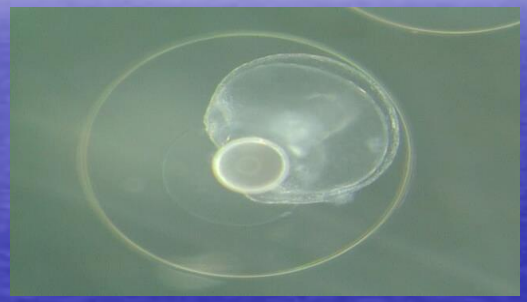
TÓXICO	HUEVOS	LARVAS	
	LC_{50} (48 horas)	LC_{50} (48 horas)	LC_{50} (96 horas)
COBRE	**0.054**	0.261	0.064
LINDANO	0.578	0.359	**0.025**
AGUA RESIDUAL	0.132	---	0.037

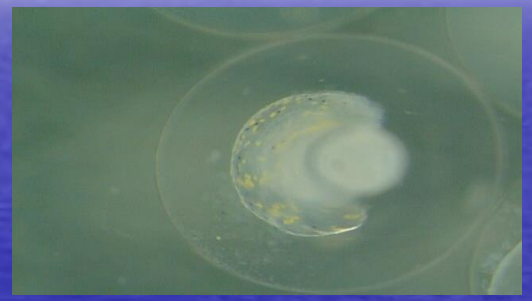
MORPHOLOGICAL ALTERATIONS IN GILTHEAD EGGS

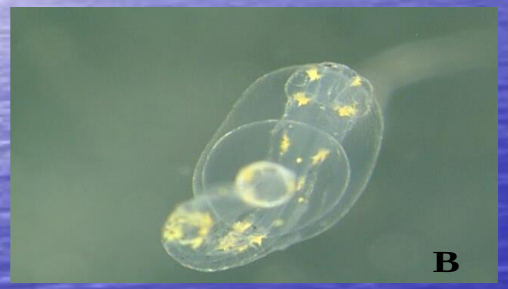


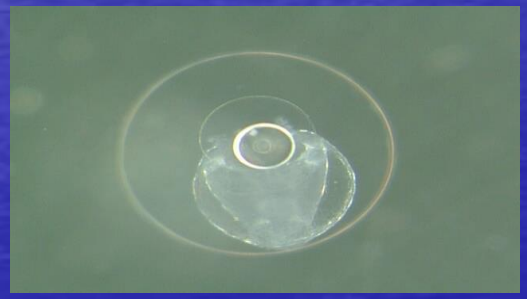


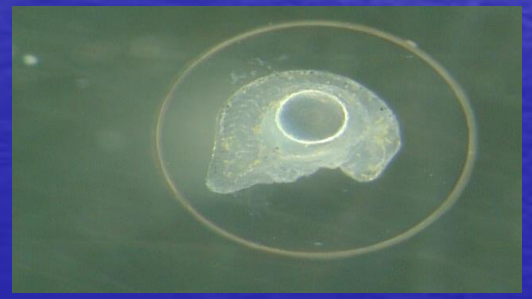


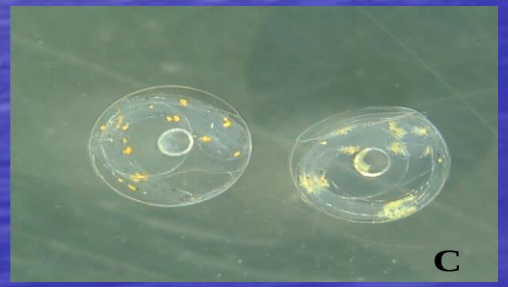












0,025 mg/L Cu

0,050 mg/L Cu

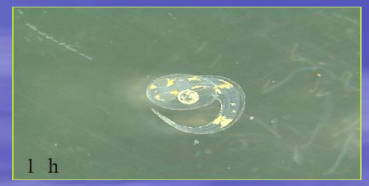
0,075 mg/L Cu

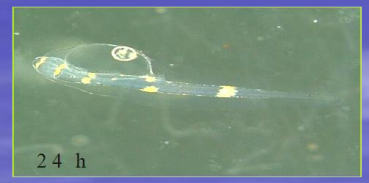
72 Hours

24 Hours

48 Hours

MORPHOLOGICAL ALTERATIONS IN LARVAE OF GILTHEAD





24 Hours

0.1 mg/L Cu

0.5 mg/L Cu

0.1 mg/L Cu

0.25 mg/L Cu

72 Hours

24 Hours

0.1 mg/L Lindano

5 mg/L Lindano

0.1 mg/L Lindano

5 mg/L Lindano

72 Hours

24 Hours

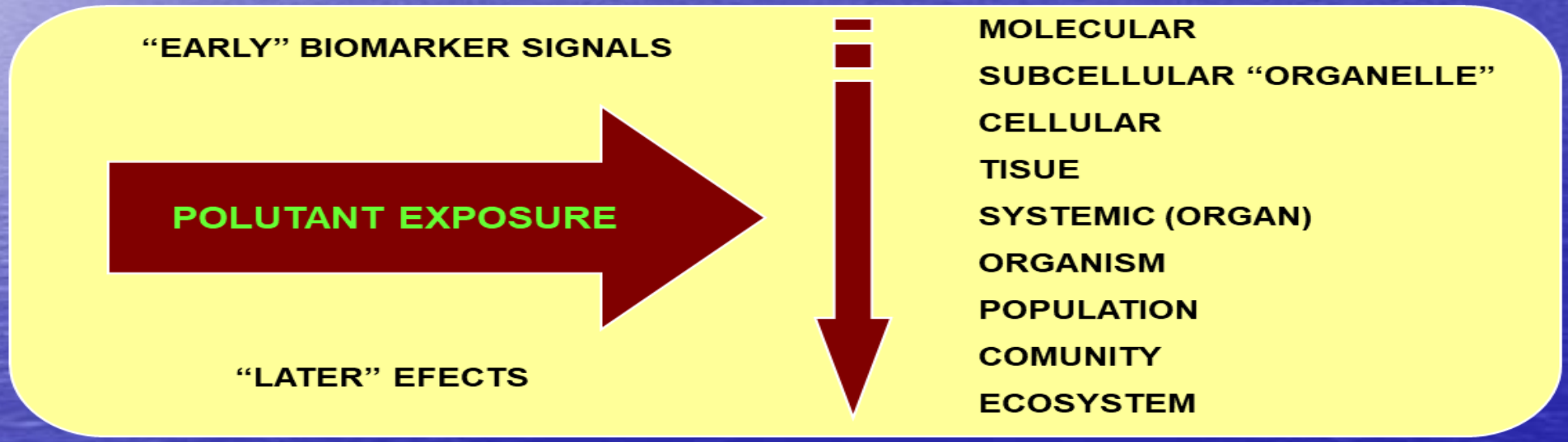
1/1000 Wastewate

1/10 Wastewater

96 Hours

- **Biomarkers**

A **BIOMARKER** is defined as a change in a biological response (ranging from molecular through cellular and physiological responses to behavioral changes) which can be related to exposure to or toxic effects of environmental chemicals (Peakall, 1994).



The ideal biomarkers should be early detected and be able to show adverse effects before they are irreversible.

Biomarkers of exposure

Covering the detection and measurement of an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism.

Biomarkers of effect

Including measurable biochemical, physiological or other alterations within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease.

Biomarkers of susceptibility

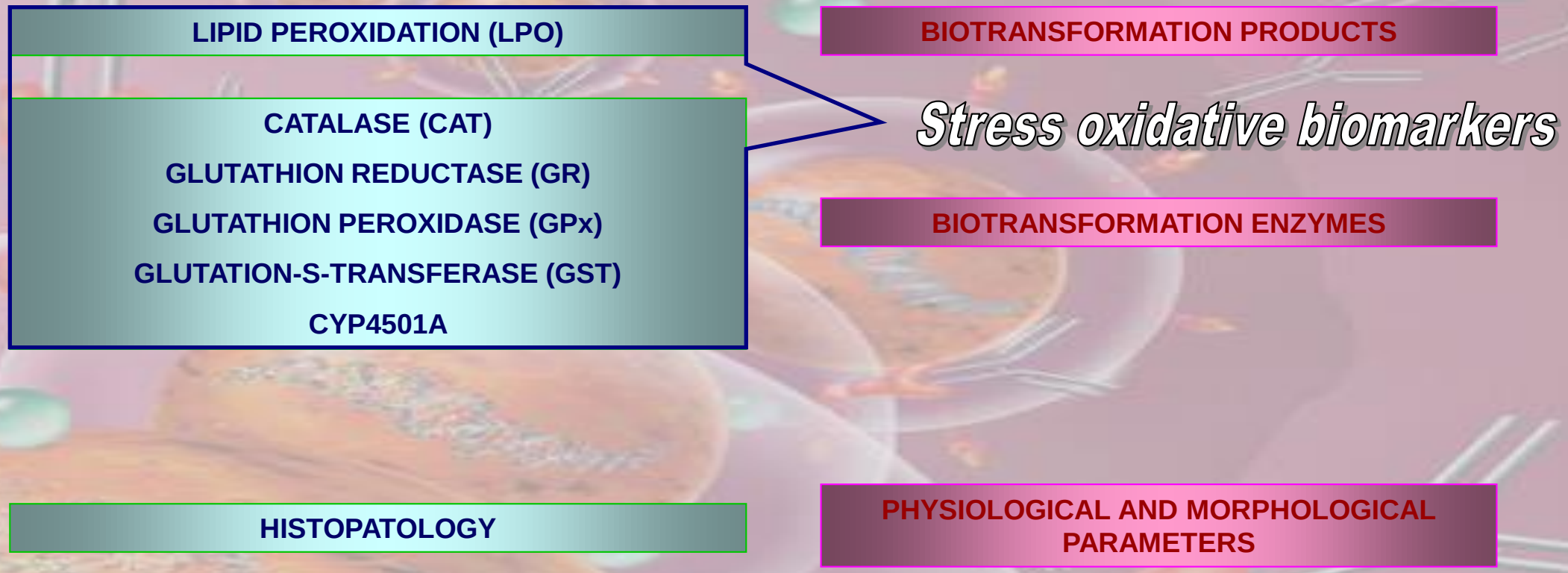
Indicating the inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic sub-stance, including genetic factors and changes in receptors which alter the susceptibility of an organism to that exposure.

EXPOSITION BIOMARKERS

EFFECT BIOMARKERS

SUSCEPTIBILITY BIOMARKERS

- **Some effect and exposure biomarkers**



- **Stress oxidative biomarkers**

Reactive oxygen species (ROS)

Stressors

ROS: OH^-, O_2^-, H_2O_2

Oxidative Stress

Molecular damage

- Lipids and Fatty Acids
- Aminoacids
- Proteins
- Nucleic Acids
- Pigments

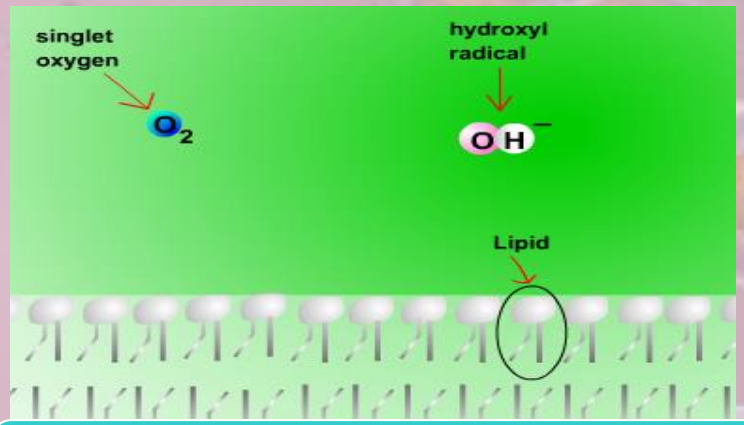
Cellular effects

- Membrane Damage
- Loss of organelle functions
- Reduction of metabolic efficiency
- Reduced carbon fixation
- Electrolite leakage
- Chromatid breaks
- Mutations

Cell Death

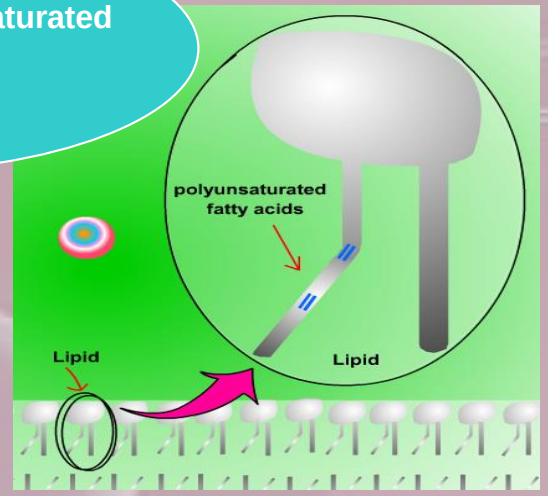
- **Stress oxidative biomarkers**

Lipid Peroxidation (LPO)

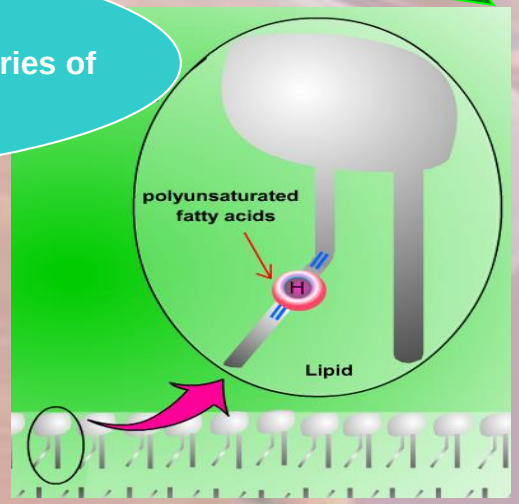


Reactive molecules will iniciate lipid peroxidation

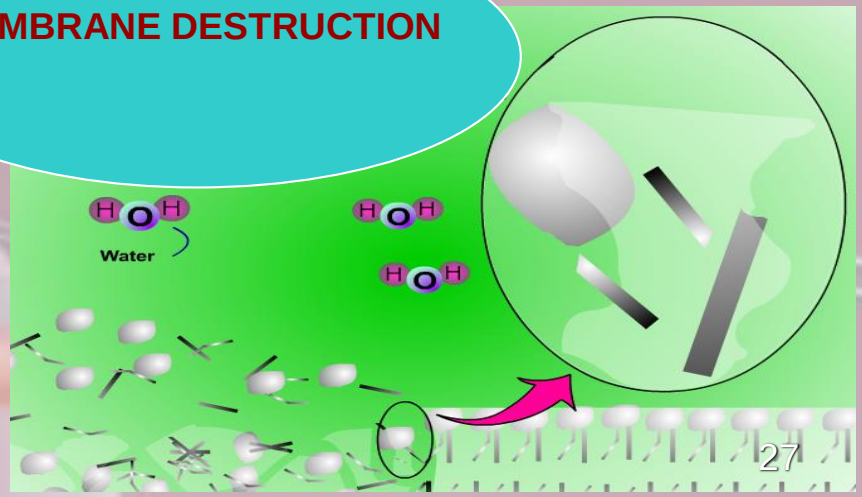
LPO occurs in polyunsaturated fatty acids



Hydrogen extraction from the lipid initiating a series of reactions

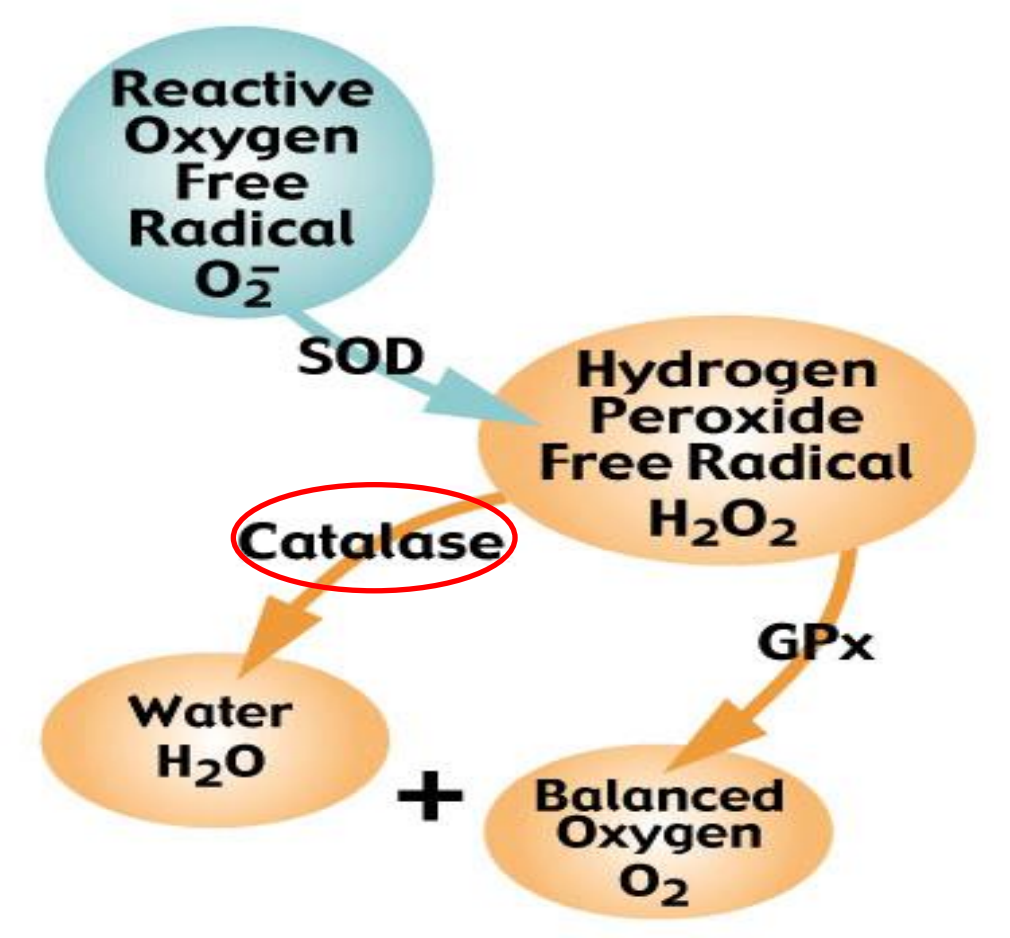


MEMBRANE DESTRUCTION



- Stress oxidative biomarkers

Catalase (CAT)

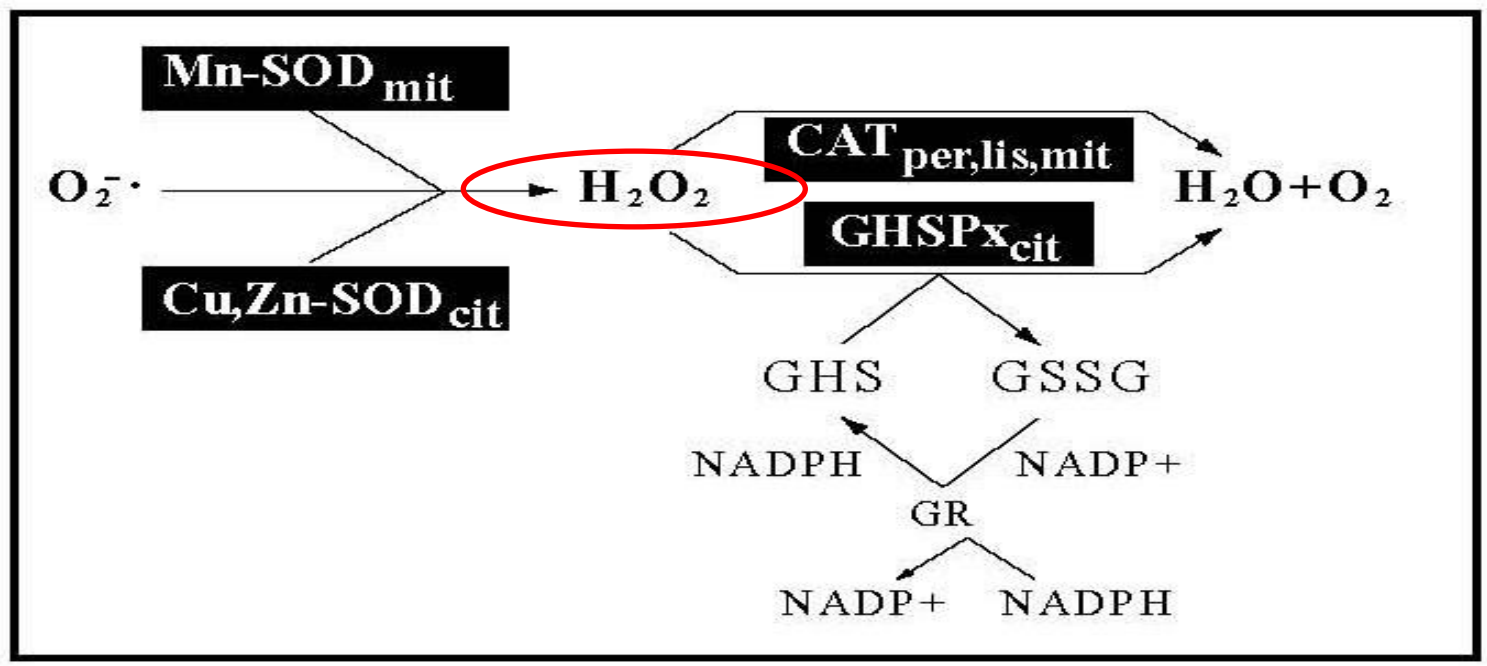


Catalyze the decomposition of hydrogen peroxide to water and oxygen.

$$H_2O_2 + Fe(III)\text{-}E \rightarrow H_2O + O{=}Fe(IV)\text{-}E$$

$$H_2O_2 + O{=}Fe(IV)\text{-}E \rightarrow H_2O + Fe(III)\text{-}E + O_2$$

- **Stress oxidative biomarkers** **Glutathione peroxidase (GPx)**

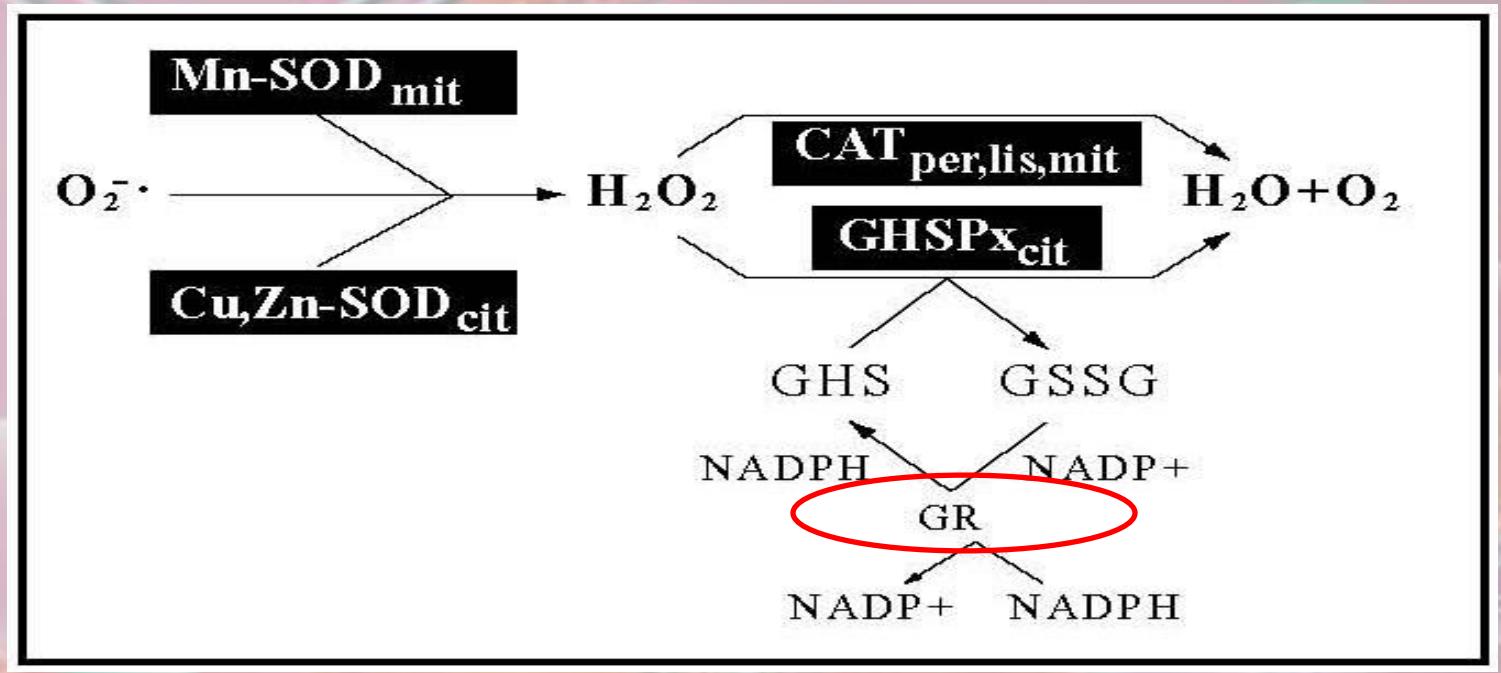


Reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.

GPx

$2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$

- **Stress oxidative biomarkers** **Glutathione reductase (GR)**



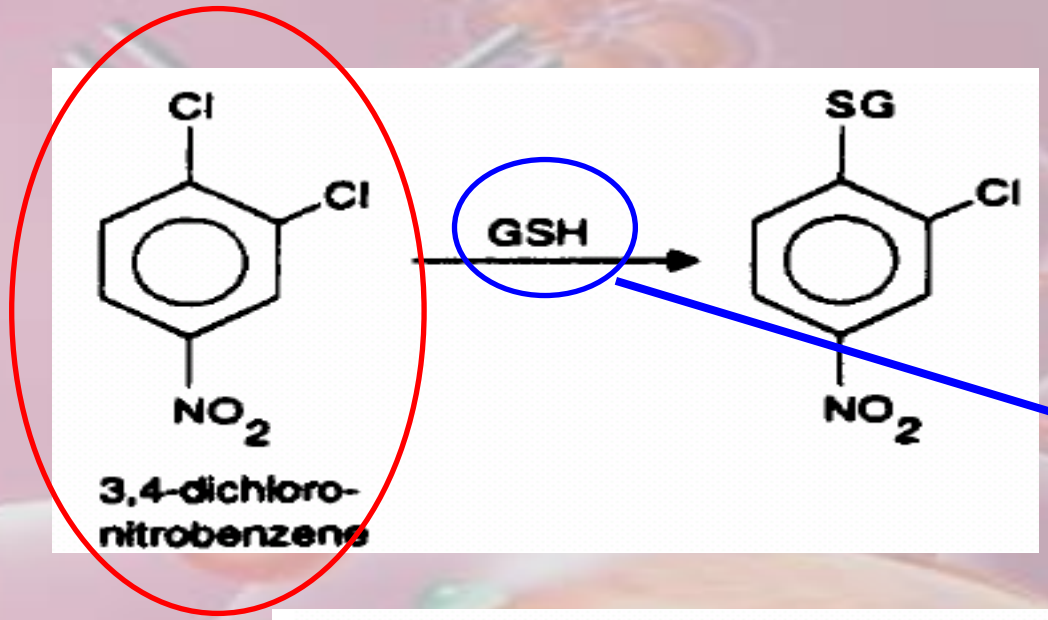
Maintaning GSH/GSSG homeostasis

2GSH + H2O2 → GS–SG + 2H2O

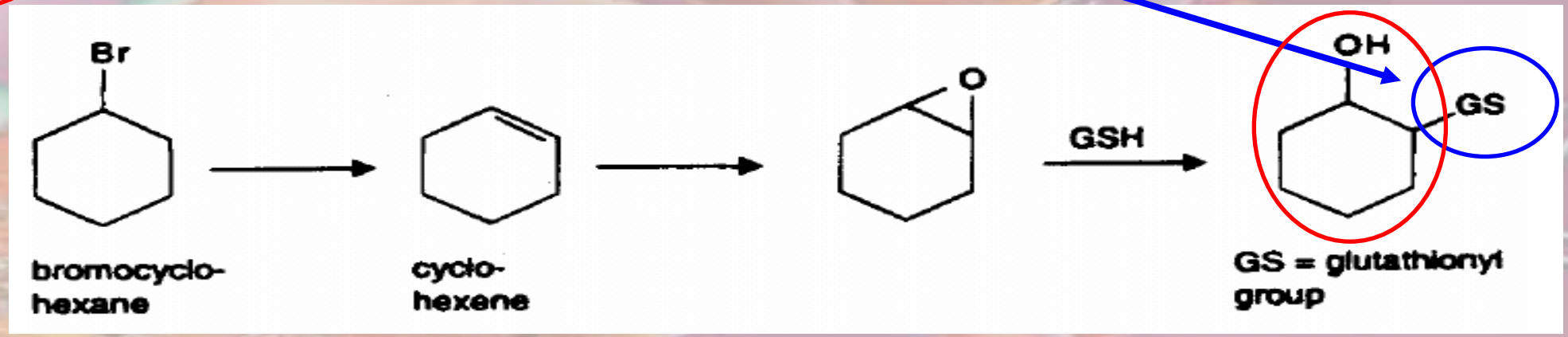
GS–SG + NADPH + H+ → 2 GSH + NADP+

GR

- **Stress oxidative biomarkers** **Glutathione transpherase (GST)**

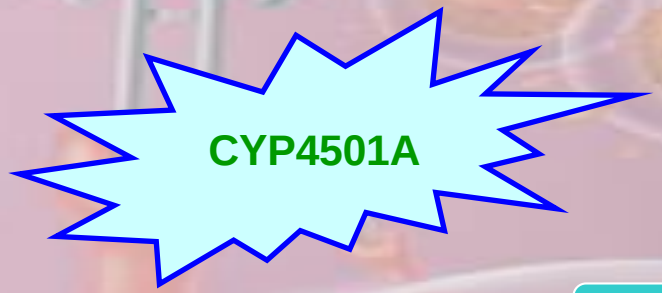


Conjugating of compounds with reduced glutathione.



- **Biomarkers**

CYP4501A



EXOGEN AND ENDOGEN CHEMICALS

APOLAR CHEMICALS

Phase I

POLAR ACTIVE CHEMICALS

TRANSFORMED CHEMICALS

Phase II

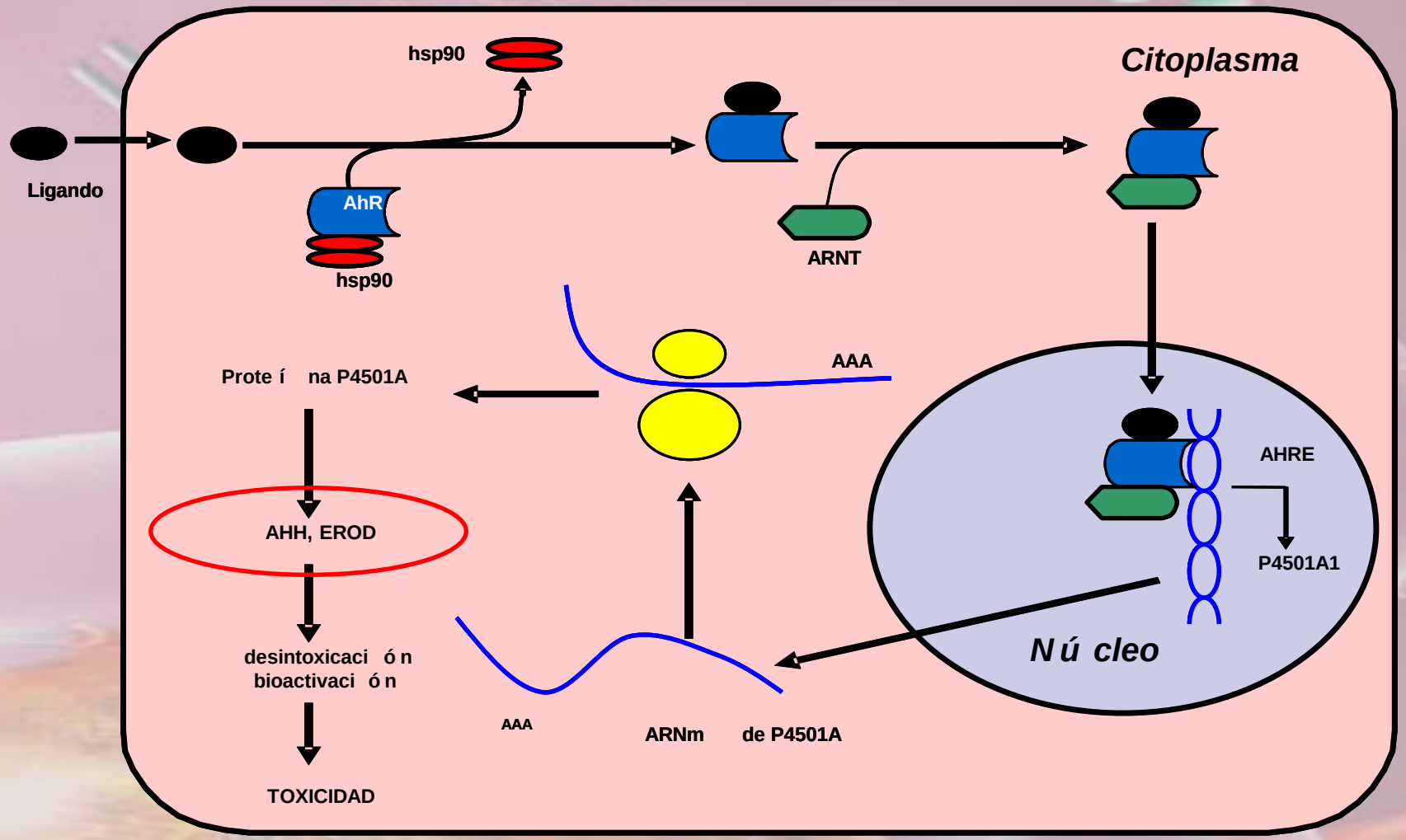
CONJUGATED

POLAR INACTIVE CHEMICALS

EXCRETION

- **Biomarkers**

CYP4501A



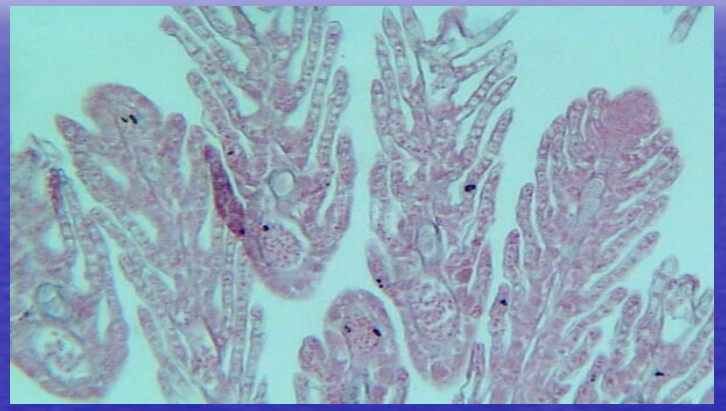
hsp90: protein"heat shock 90".
AhR: aryl- hidrocarbon receptor.
ARNT: translocator protein of aryl-hydrocarbon receptor.
AHRE: response elements to aryl-hydrocarbon receptor.
AAA: aminoacids

Ethoxyresorufin-*O*-deethylase (EROD) acts as a substrate for CYP1A1 and measurement of ethoxyresorufin O-deethylase provides a more direct method of detection for this enzyme.

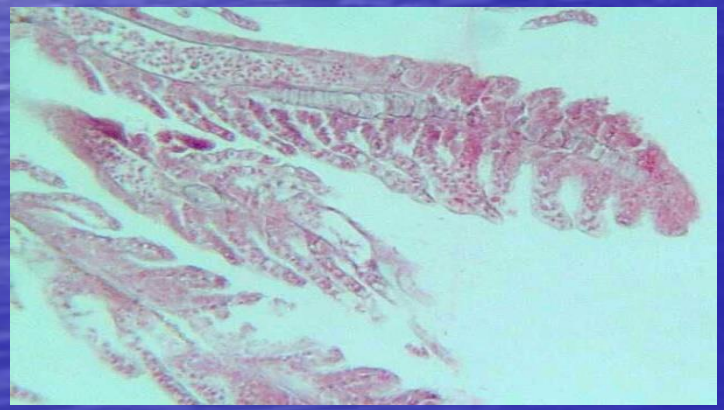
HISTOPATHOLOGICAL ANALYSIS IN FISH TISSUES

GILLS

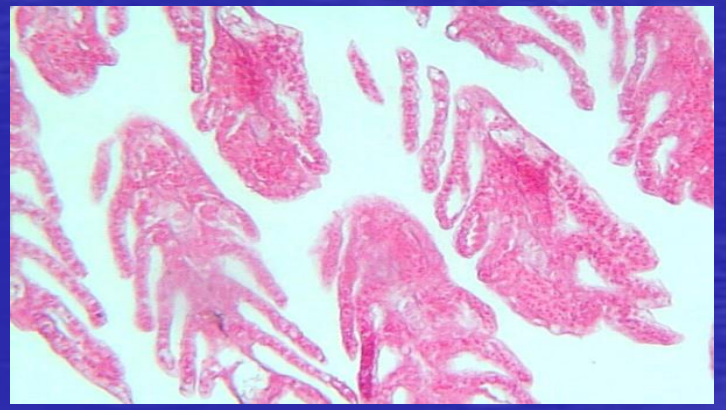
CONTROL



0.01 mg/l Cu^{2+}



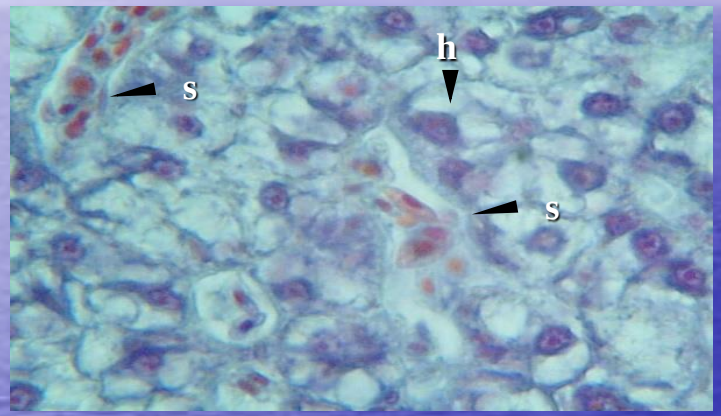
0.1 mg/l Cu^{2+}



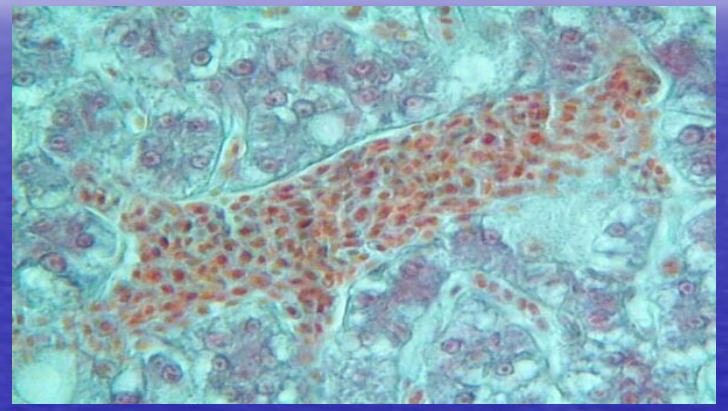
1 mg/l Cu^{2+}

HISTOPATHOLOGICAL ANALYSIS IN FISH TISSUES

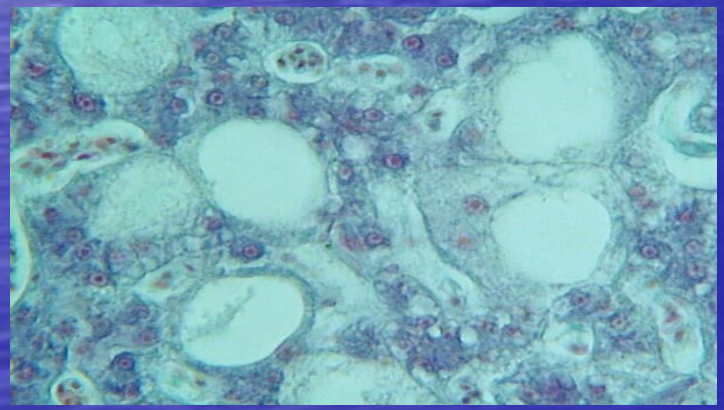
LIVER



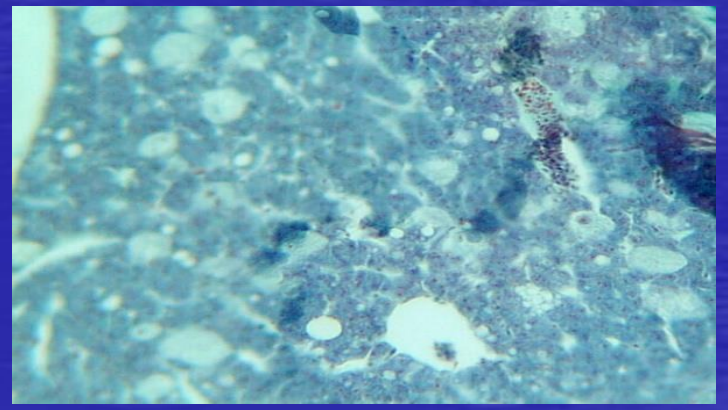
CONTROL



0.01 mg/l Cu^{2+}



0.1 mg/l Cu^{2+}



1 mg/l Cu^{2+}

¡GRACIAS! Thank you Faleminderit Hvala.

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OTHER INFORMATION:
[Links to the oficial web of the master or personal information]