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UNIVERSITY OF LISBON
INTERDISCIPLINARY STUDIES
ON SUSTAINABLE ENVIRONMENT AND SEAS

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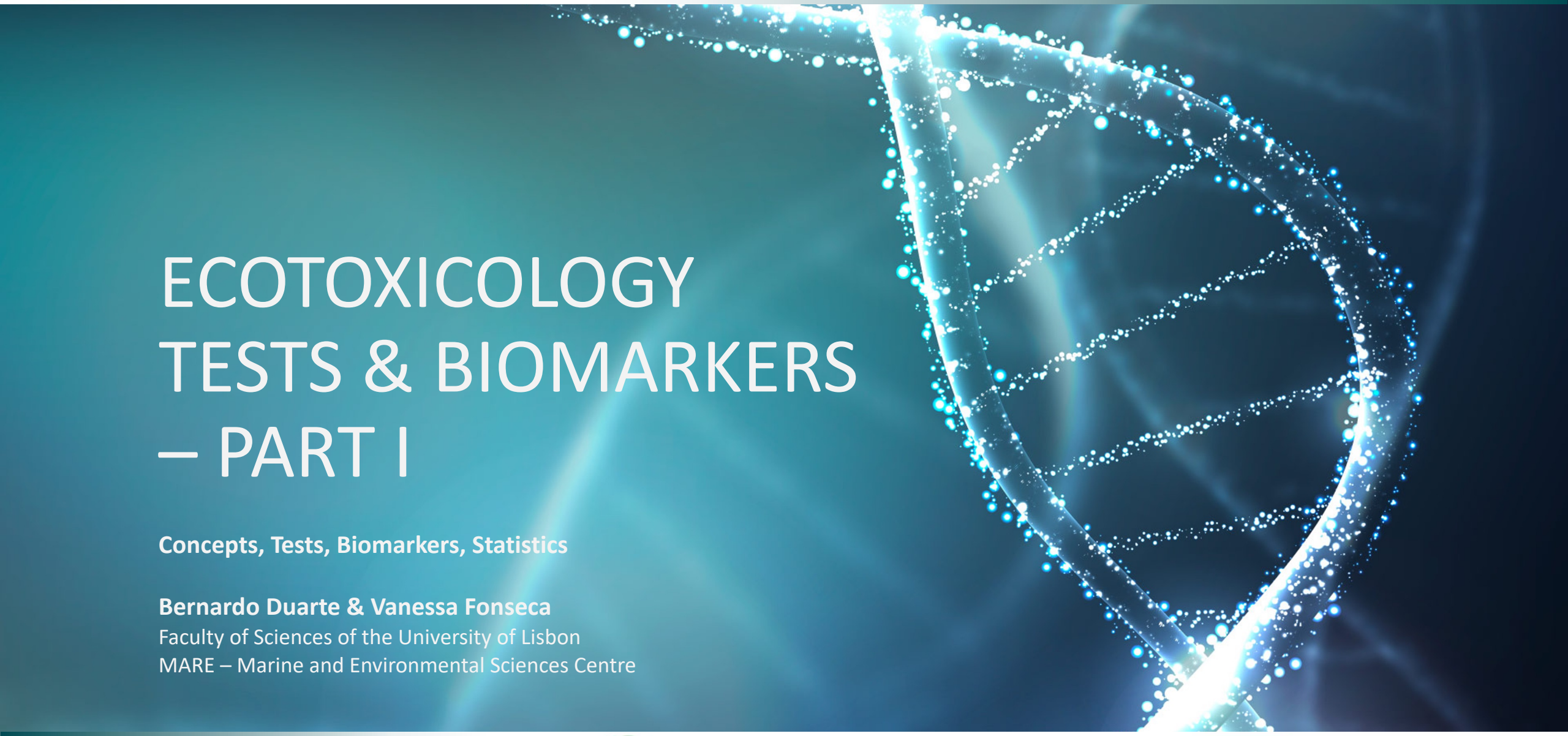

ECOTOXICOLOGY TESTS & BIOMARKERS – PART I

Concepts, Tests, Biomarkers, Statistics

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TESTS

Trial preparation, setups and typologies

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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.

ECOTOXICITY TESTS

Typologies

- **Acute**: 1-4 days (at least 10% of the organism life cycle)
- **Subacute**: standard 28 days test
- **Subchronic**: standardized to 90 days
- **Chronic**: more than 90 days (should allow a complete life cycle)
- **Transgenerational**: Allows production of a new generation and evaluates the effects on the offspring.

Typology

The exposure typology should reflect the environmental exposure time typically observed or to answer to the target scientific question (for e.g. what is the effect at the reproduction level).

ORGANIMS

- Bacteria
- Micro- and macro- algae
- Plants
- Invertebrates
- Fishes
- Mammals

ORGANISM

The selected organism should be cosmopolitan and representative of a certain group/environment.

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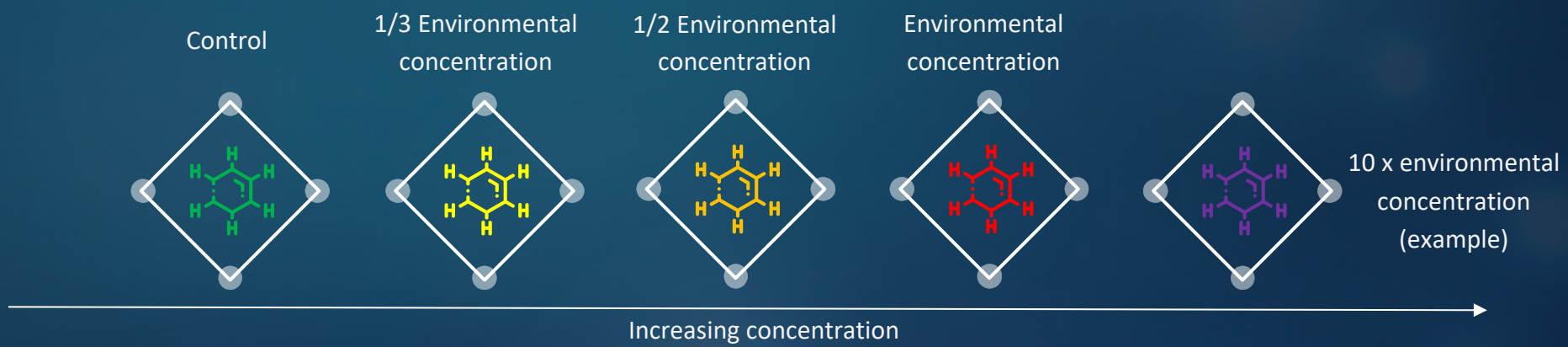
ECOTOXICITY TESTS

INDIVIDUALS

- Clonal or with the same genetic background (lab cultured/maintained organisms);
- Similar age or life cycle stage;
- Similar sex (or grouped by sex if sex is a variable to analyse);
- Similar morphometric characteristics (height, weight, volume);
- Similar life history (maintained or reproduced under the same abiotic conditions);

DESIGN

- Control and test groups exposed simultaneously and under the same environmental conditions (light, temperature, etc);
- A consistent number of replicates must be ensure in all exposure mesocosmos;
- Increasing concentrations should follow a mathematical and logic succession or increase rate;



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ECOTOXICITY TESTS

DECONTAMINATION

- In Ecotoxicology Decontamination refers to the **cleaning** of laboratory materials (glass and plasticware) from **contaminants and chemical agents**, avoiding these to contaminate the exposure trial.
- Typically uses acid or alkaline detergents, followed by acid bath and acetone (or other polar solvent) washing.
- Removes all adsorbed ions and molecules.

STYERILIZATION

- Any process that eliminates, **removes, kills, or deactivates all forms of life**.
- For most ecotoxicological trials is not a requirement although it is advisable.

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PARAMETERIZATION

Ecotoxicity parameters, calculations and significance

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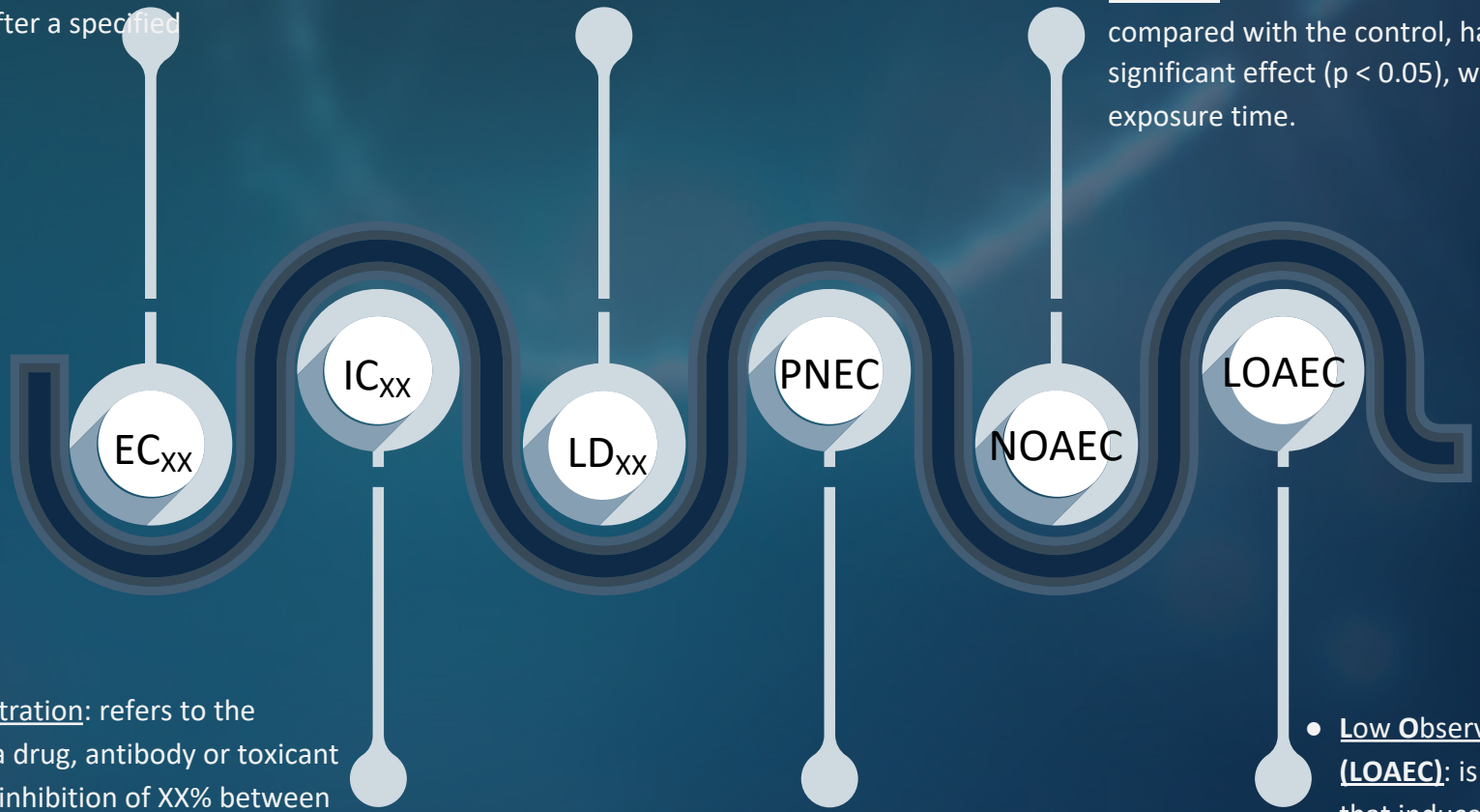
ECOTOXICITY TESTS

- Effective Concentration: refers to the concentration of a drug, antibody or toxicant which induces a response of XX% between the baseline and maximum after a specified exposure time.

- Lethal Concentration: of a toxin, radiation, or pathogen is the dose required to kill XX% of the members of a tested population after a specified test duration.

- No Observed Adverse Effect Concentration (NOAEC): is the tested concentration which, when compared with the control, has no statistically significant effect ($p < 0.05$), within a given exposure time.



- Inhibitory Concentration: refers to the concentration of a drug, antibody or toxicant which induces an inhibition of XX% between the baseline and maximum after a specified exposure time.

- Predicted No Effect Concentration (PNEC): the concentration of a chemical which marks the limit at which below no adverse effects of exposure in an ecosystem are measured.

- Low Observed Adverse Effect Concentration (LOAEC): is the lowest tested concentration that induces a response significantly different from control..

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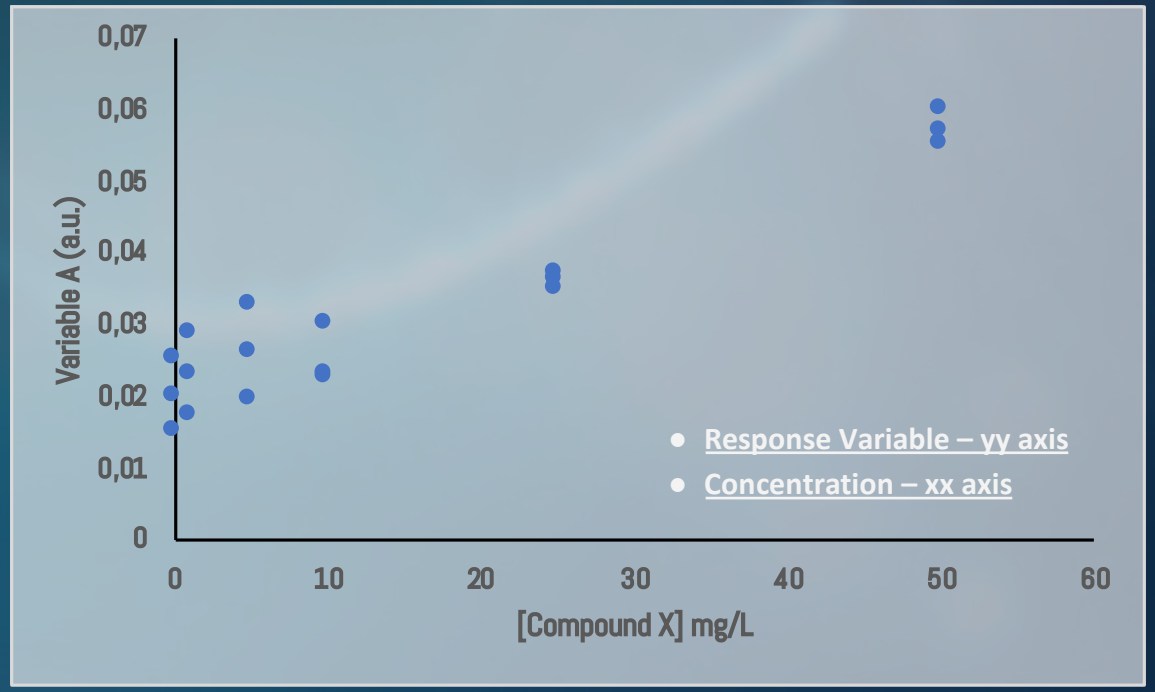
[Compound X] mg/L	Variable A (a.u.)
0	0,02
0	0,015
0	0,025
1	0,023
1	0,01725
1	0,02875
5	0,026
5	0,0195
5	0,0325
10	0,03
10	0,0225
10	0,023
25	0,035
25	0,037
25	0,036
50	0,06
50	0,057
50	0,055



Plotting the response of a certain biomarker against the exposure dose to which the organism was exposed allows to evaluate the tendency of the effect of the compound towards the target biomarker.

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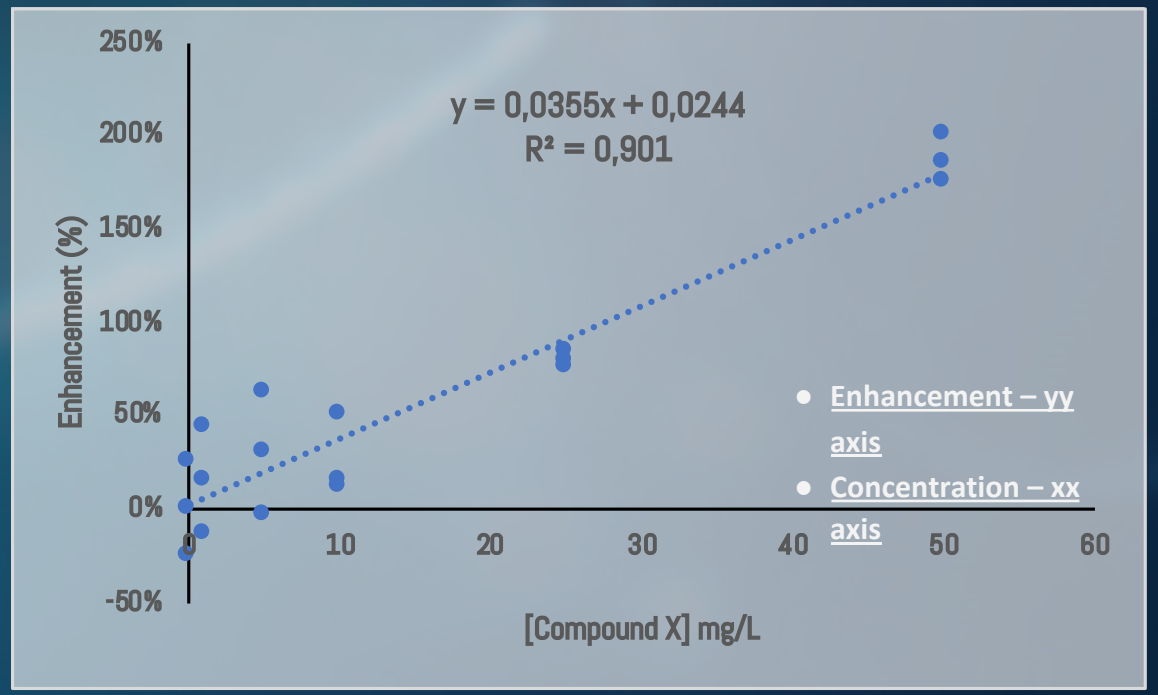
02 PARAMETERIZATION : Effective Concentration

[Compound X] mg/L	Variable A (a.u.)	Enhancement (%)
0	0,02	0%
0	0,015	-25%
0	0,025	25%
1	0,023	15%
1	0,01725	-14%
1	0,02875	44%
5	0,026	30%
5	0,0195	-3%
5	0,0325	63%
10	0,03	50%
10	0,0225	13%
10	0,023	15%
25	0,035	75%
25	0,037	85%
25	0,036	80%
50	0,06	200%
50	0,057	185%
50	0,055	175%

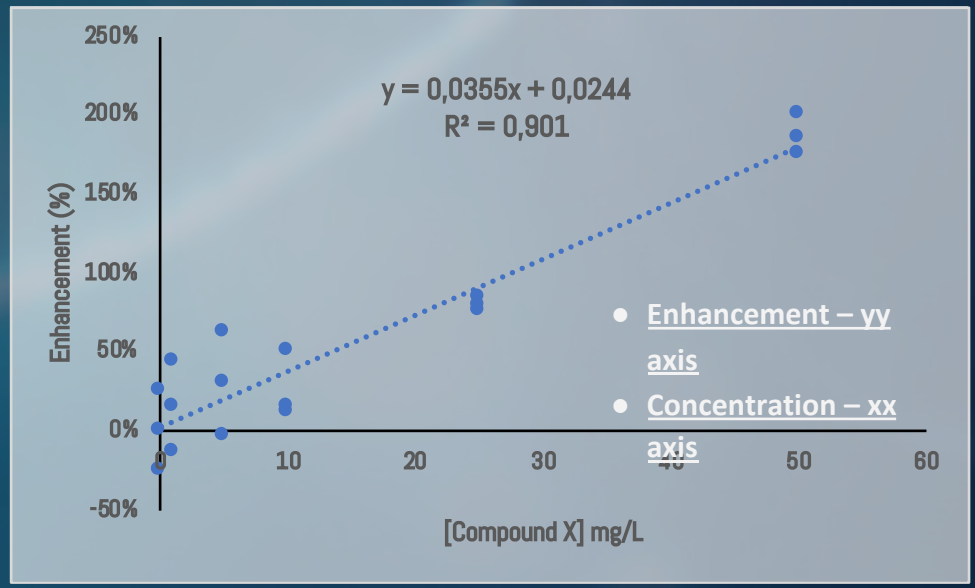
$$\text{Enhancement (\%)} = \frac{\text{Test} - \overline{\text{Control}}}{\overline{\text{Control}}}$$



A Relative Enhancement can be calculated with the above formula and a linear correlation can be plotted.

[Compound X] mg/L	Variable A (a.u.)	Enhancement (%)
0	0,02	0%
0	0,015	-25%
0	0,025	25%
1	0,023	15%
1	0,01725	-14%
1	0,02875	44%
5	0,026	30%
5	0,0195	-3%
5	0,0325	63%
10	0,03	50%
10	0,0225	13%
10	0,023	15%
25	0,035	75%
25	0,037	85%
25	0,036	80%
50	0,06	200%
50	0,057	185%
50	0,055	175%

$$\text{Enhancement (\%)} = \frac{\text{Test} - \overline{\text{Control}}}{\overline{\text{Control}}}$$



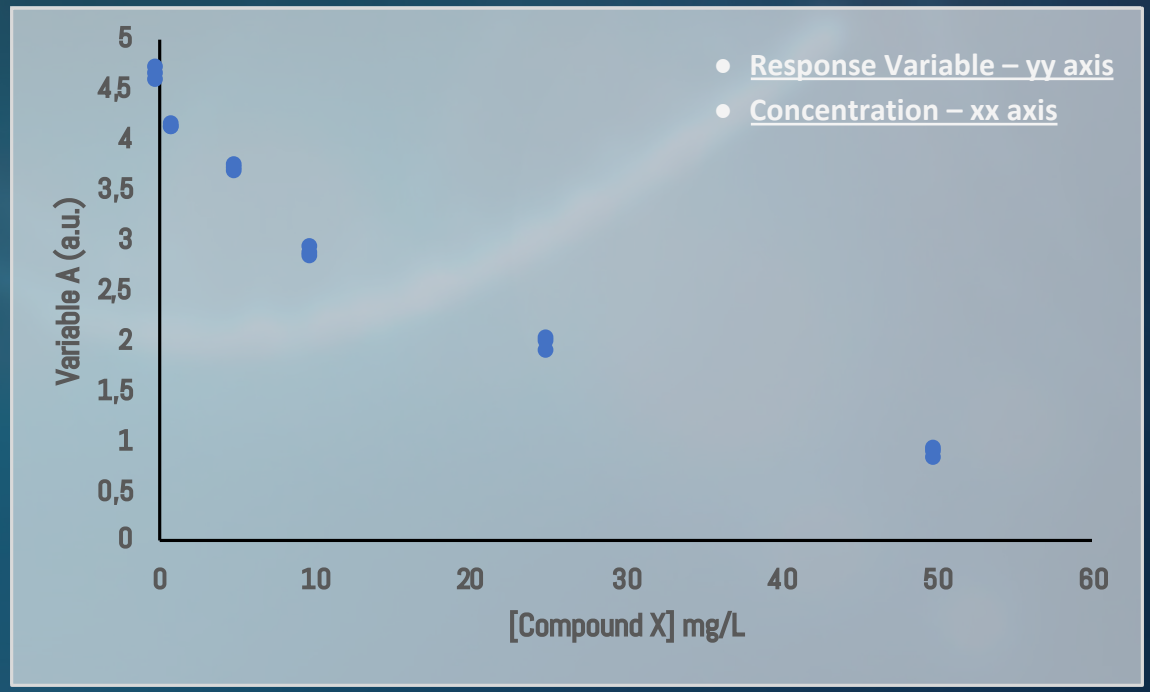
Using the linear regression equation calculate the concentration at which the enhancement is 50% (EC_{50})

$50\% = 0.0355x + 0.0244 \Leftrightarrow 0.5 = 0.0355x + 0.0244 \Leftrightarrow 0.5 - 0.0244 = 0.0355x$

$x = 13.40$ mg/L = EC_{50}

Upon the application of 13.40 mg/L the variable A suffers a 50% increase relative to the control.

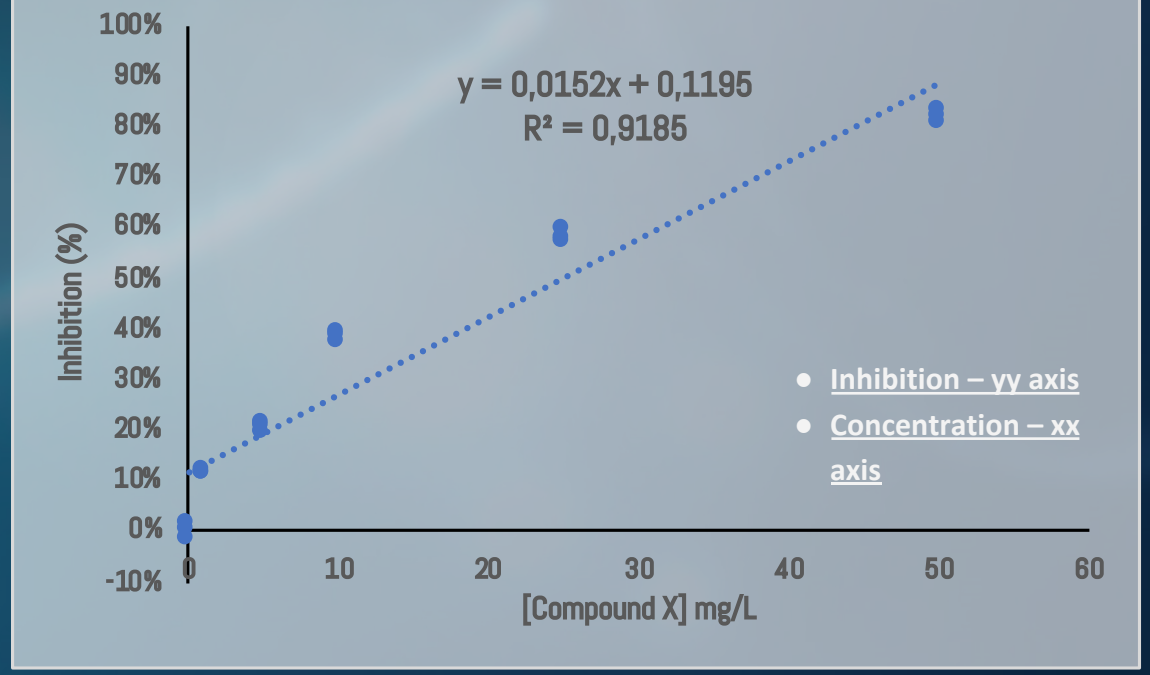
[Compound X] mg/L	Variable A (a.u.)
0	4,69
0	4,57
0	4,63
1	4,08
1	4,11
1	4,095
5	3,72
5	3,65
5	3,685
10	2,89
10	2,81
10	2,85
25	1,99
25	1,87
25	1,95
50	0,9
50	0,79
50	0,845



Plotting the response of a certain biomarker against the exposure dose to which the organism was exposed allows to evaluate the tendency of the effect of the compound towards the target biomarker. In this case an inhibition.

[Compound X] mg/L	Variable A (a.u.)	Inhibition (%)
0	4,69	-1%
0	4,57	1%
0	4,63	0%
1	4,08	12%
1	4,11	11%
1	4,095	12%
5	3,72	20%
5	3,65	21%
5	3,685	20%
10	2,89	38%
10	2,81	39%
10	2,85	38%
25	1,99	57%
25	1,87	60%
25	1,95	58%
50	0,9	81%
50	0,79	83%
50	0,845	82%

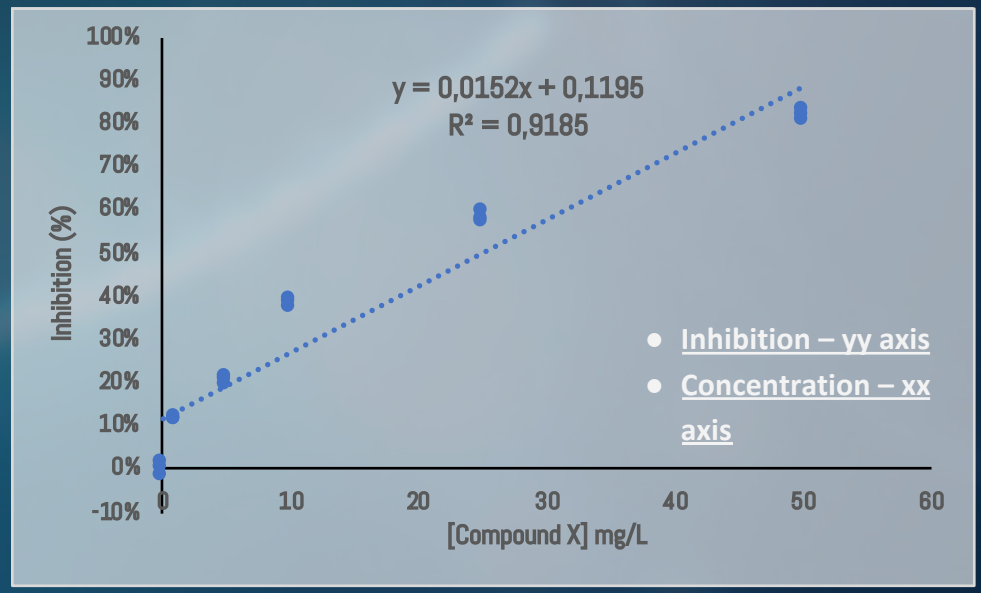
$$\text{Inhibition (\%)} = \frac{\overline{\text{Control}} - \text{Test}}{\overline{\text{Control}}}$$



A Relative Inhibition can be calculated with the above formula and a linear correlation can be plotted.

[Compound X] mg/L	Variable A (a.u.)	Inhibition (%)
0	0,02	0%
0	0,015	-25%
0	0,025	25%
1	0,023	15%
1	0,01725	-14%
1	0,02875	44%
5	0,026	30%
5	0,0195	-3%
5	0,0325	63%
10	0,03	50%
10	0,0225	13%
10	0,023	15%
25	0,035	75%
25	0,037	85%
25	0,036	80%
50	0,06	200%
50	0,057	185%
50	0,055	175%

$$\text{Inhibition (\%)} = \frac{\overline{\text{Control}} - \text{Test}}{\overline{\text{Control}}}$$



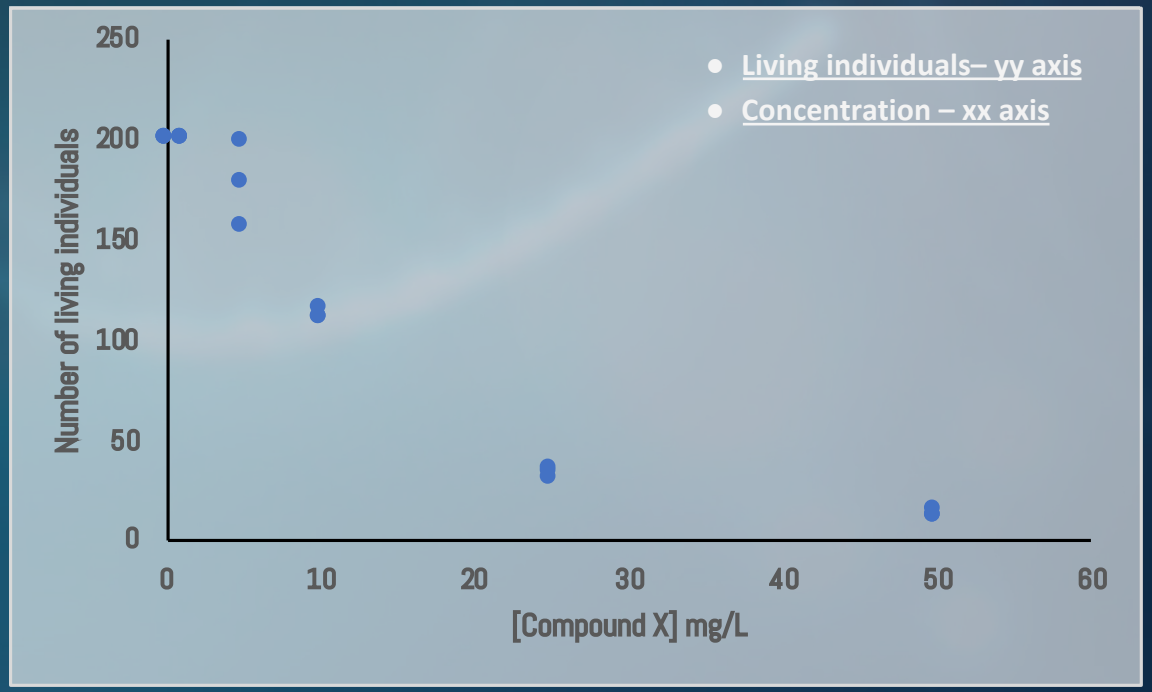
Using the linear regression equation calculate the concentration at which the inhibition was 50% (IC_{50})

$50\% = 0.0152x + 0.1195 \Leftrightarrow 0.5 = 0.0152x + 0.1195 \Leftrightarrow 0.5 - 0.1195 = 0.0152x$

$x = 13.40$ mg/L = IC_{50}

Upon the application of 13.40 mg/L the variable A suffers a 50% inhibition relative to the control.

[Compound X] mg/L	Number of living individuals
0	200
0	200
0	200
1	200
1	200
1	200
5	156
5	178
5	198
10	110
10	115
10	111
25	30
25	33
25	35
50	11
50	12
50	14



Plotting the response of the number of living organisms against the exposure dose to which the organism was exposed allows to evaluate the mortality tendency of the compound in the test organism.

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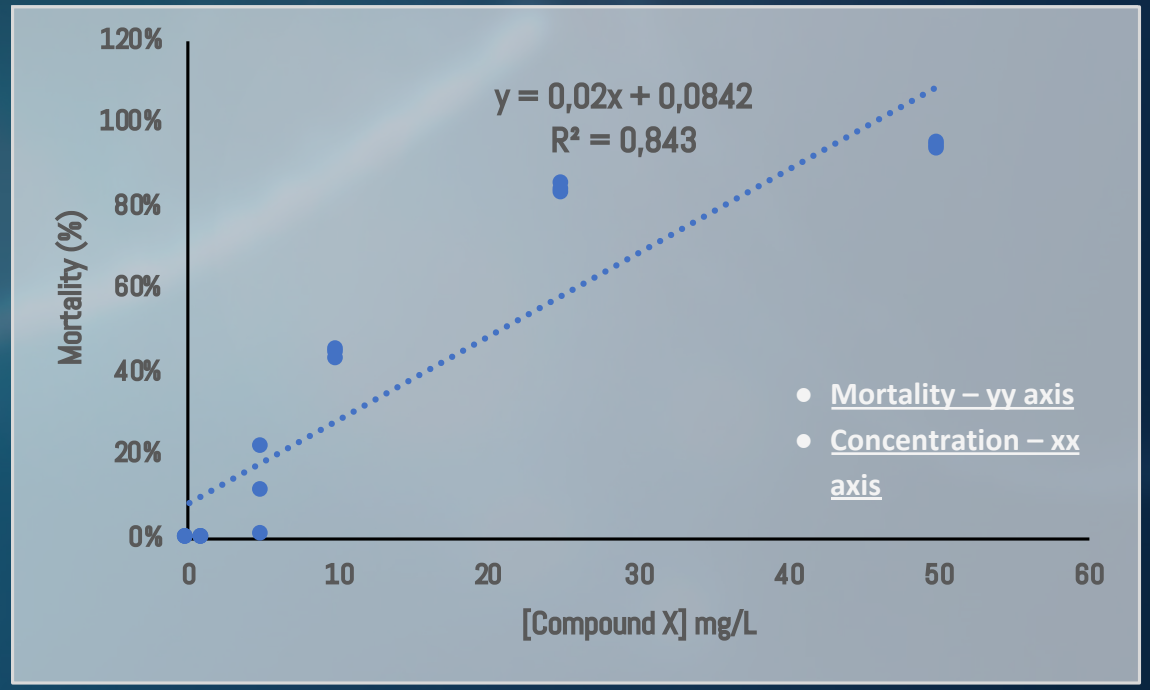
02 PARAMETERIZATION : LETHAL Concentration

[Compound X] mg/L	Variable A (a.u.)	Mortality (%)
0	4,69	0%
0	4,57	0%
0	4,63	0%
1	4,08	0%
1	4,11	0%
1	4,095	0%
5	3,72	22%
5	3,65	11%
5	3,685	1%
10	2,89	45%
10	2,81	43%
10	2,85	45%
25	1,99	85%
25	1,87	84%
25	1,95	83%
50	0,9	95%
50	0,79	94%
50	0,845	93%

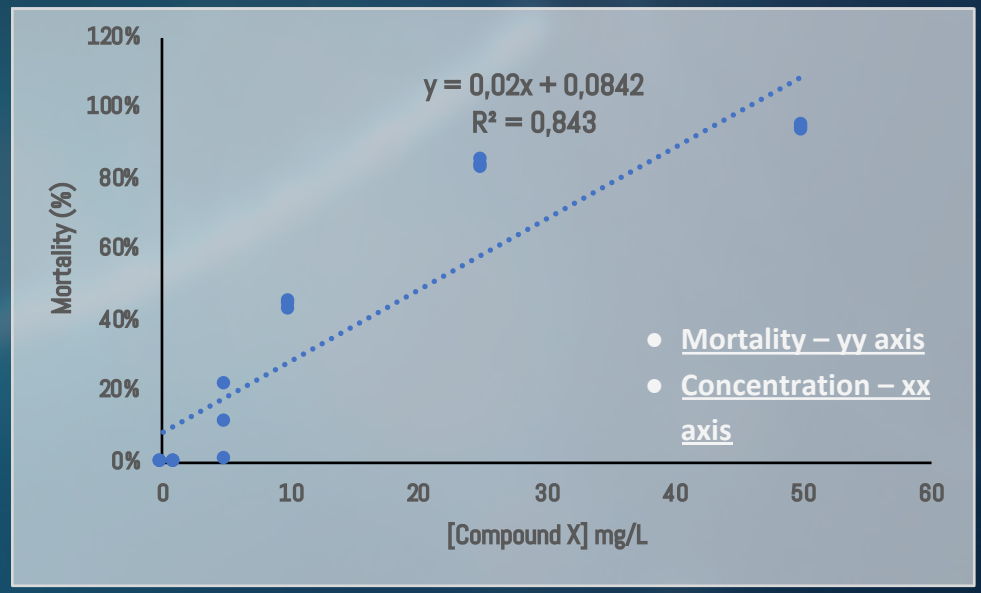
$$\text{Mortality (\%)} = \frac{\overline{\text{Control}} - \text{Test}}{\overline{\text{Control}}}$$



A Mortality percentage can be calculated with the above formula and a linear correlation can be plotted.

[Compound X] mg/L	Variable A (a.u.)	Mortality (%)
0	0,02	0%
0	0,015	-25%
0	0,025	25%
1	0,023	15%
1	0,01725	-14%
1	0,02875	44%
5	0,026	30%
5	0,0195	-3%
5	0,0325	63%
10	0,03	50%
10	0,0225	13%
10	0,023	15%
25	0,035	75%
25	0,037	85%
25	0,036	80%
50	0,06	200%
50	0,057	185%
50	0,055	175%

$$\text{Mortality (\%)} = \frac{\overline{\text{Control}} - \text{Test}}{\overline{\text{Control}}}$$



Using the linear regression equation calculate the concentration at which half the number of initial individuals is dead (LC_{50})

$50\% = 0.02x + 0.0842 \Leftrightarrow 0.5 = 0.02x + 0.0842 \Leftrightarrow 0.5 - 0.0842 = 0.02x$

$x = 15.79$ mg/L = LC_{50}

Upon the application of 15.79 mg/L 50% of the individual die.

ECOTOXICTY VARIABLES

- EC, IC and LC refer to effective, inhibitory and lethal **concentration** respectively.
- It refers to an **external or exogenous concentration** applied to a certain organism.
- Expressed as a **mass per volume unit.**

- ED, ID and LD refer to effective, inhibitory and lethal **dose** respectively.
- It refers to measured concentration **inside an organism or to an external or exogenous concentration applied to a certain organism calculated proportionally to the organism body mass**
- Expressed as **mass of target substance per organism fresh weight.**

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02 PARAMETERIZATION : VARIABLE TYPOLOGY

BEHAVIOURAL

Locomotion capacity
Feeding rates
Attack rates

MORPHOLOGICAL

Growth rates
Cell size
Tissue anomalies
Morphometric and geometry changes

METABOLIC

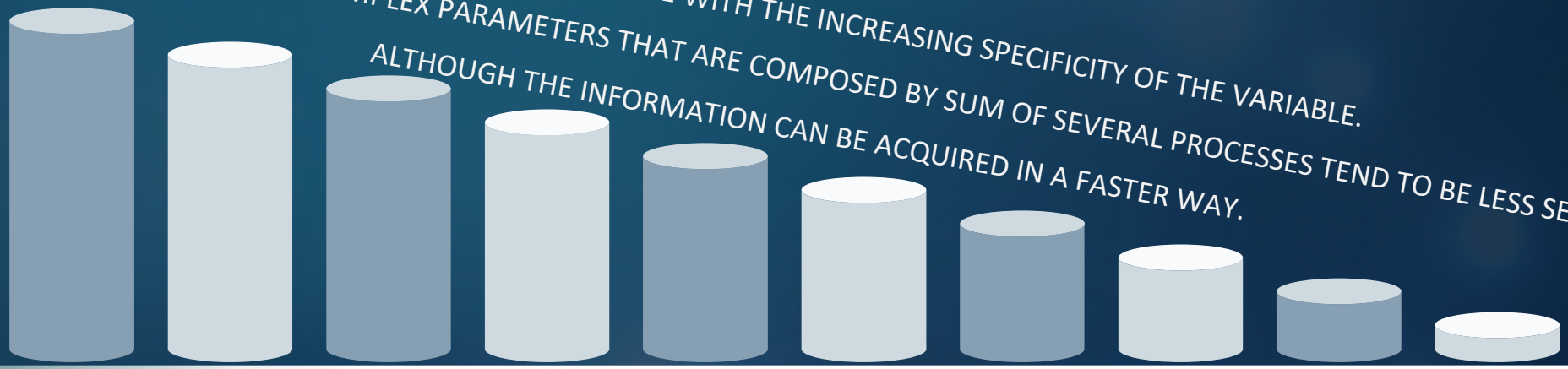
Photosynthetic activity
Respiratory activity
Sugar/Lipid consumption

MOLECULAR

Enzymatic activity
Membrane peroxidation
Protein oxidation
DNA damage
Gene expression
Metabolite production/consumption

VARIABLES THAT REFER TO COMPLEX PARAMETERS THAT ARE COMPOSED BY SUM OF SEVERAL PROCESSES TEND TO BE LESS SENSITIVE, ALTHOUGH THE INFORMATION CAN BE ACQUIRED IN A FASTER WAY.

EC AND IC TEND TO DECREASE WITH THE INCREASING SPECIFICITY OF THE VARIABLE.



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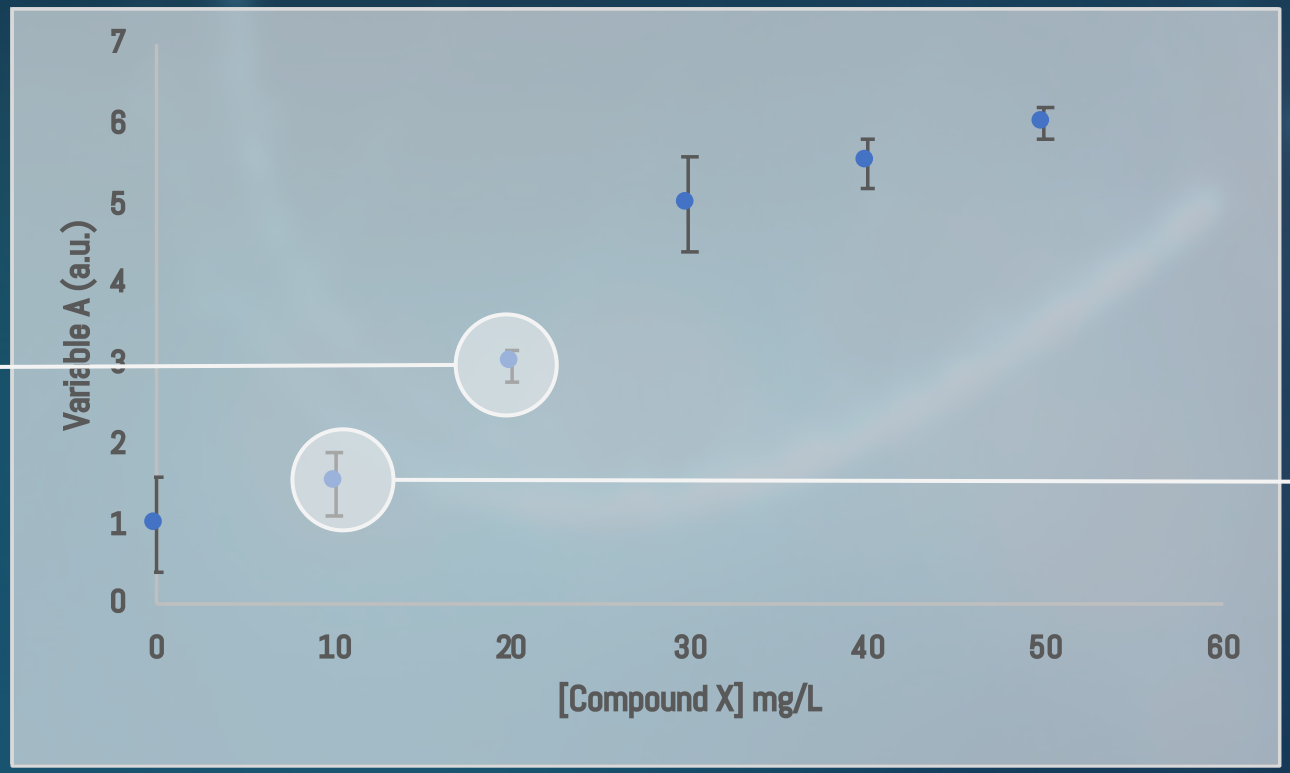
LOAEC

The lowest observed (tested) concentration that produced significant effects



NOAEC

Highest concentration at which no significant effects are still detected.

NOAEC/LOAEC versus IC/EC/LC

- NOAEC and LOAEC depend on the concentrations tested and defined by the user; if the range of concentrations tested has a low resolution power the NOAEC and LOAEC assessed can be deceiving.
- IC, EC and LC are obtained by linear regression analysis and thus even if the concentration correspondent to each of these parameters was not tested, it can be calculated.

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PREDICTED NO EFFECT CONCENTRATION (PNEC):

- The concentration of a chemical which marks the limit at which below no adverse effects of exposure in an ecosystem are measured.
- Conservative values and predict the concentration at which a chemical will likely have no toxic effect.
- Do not intended to predict the upper limit of concentration of a chemical that has a toxic effect
- PNEC values are often used in environmental risk assessment as a tool in ecotoxicology.
- A PNEC for a chemical can be calculated with acute toxicity or chronic toxicity single-species data, species sensitivity distribution (SSD) multi-species data, field data or model ecosystems data. depending of the type of data used, an **ASSESSMENT FACTOR** is used to account for the confidence of the toxicity data being extrapolated to an entire ecosystem.

Available test result	Assessment factor
One long-term test (NOEC or EC10)	100
Two long-term tests (NOEC or EC10) with species representing different living and feeding conditions	50
Three long-term tests (NOEC or EC10) with species representing different living and feeding conditions	10

PREDICTED NO EFFECT CONCENTRATION (PNEC):

- The concentration of a chemical which marks the limit at which below no adverse effects of exposure in an ecosystem are measured.
- Conservative values and predict the concentration at which a chemical will likely have no toxic effect.
- Do not intended to predict the upper limit of concentration of a chemical that has a toxic effect
- PNEC values are often used in environmental risk assessment as a tool in ecotoxicology.
- A PNEC for a chemical can be calculated with acute toxicity or chronic toxicity single-species data, species sensitivity distribution (SSD) multi-species data, field data or model ecosystems data. depending of the type of data used, an **ASSESSMENT FACTOR** is used to account for the confidence of the toxicity data being extrapolated to an entire ecosystem.

- The ASSESSMENT FACTOR can be determined by the available bibliography or pre-defined taking into account the type of assay.

BIBLIOGRAPHIC INFORMATION AVAILABILITY

Available test result	Assessment factor
One long-term test (NOEC or EC10)	100
Two long-term tests (NOEC or EC10) with species representing different living and feeding conditions	50
Three long-term tests (NOEC or EC10) with species representing different living and feeding conditions	10

PRE-DEFINED BY THE TEST TYPE

Available test result	Assessment factor
Acute Toxicity Data	The lowest LC50 in the compiled database is then divided by the assessment factor to calculate the PNEC for that data. The assessment factor applied to acute toxicity data is typically 1000.
Chronic Toxicity Data	The lowest NOEC value in the test dataset is divided by an assessment factor between 10 and 100 dependent on the diversity of test organisms and the amount of data available. If there are more species or data, the assessment factor is lower.

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