How to quantify the sensitivity of the Daphnia toximeter? Behavioural analysis on *Daphnia magna* exposed to different substances

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INTRODUCTION

Online biomonitoring are used with succes for

- drinking water distribution intake

- antiterrorism chemical weapons control

EDF R&D is working on testing biomonitors for quality control of surface water

I. INTRODUCTION

MIRE station designed by INERIS & EDF :

MIRE = Module Integrator of Environmental sewage

- Algae toximeter 
- Daphnia toximeter 
- Fish toximeter 

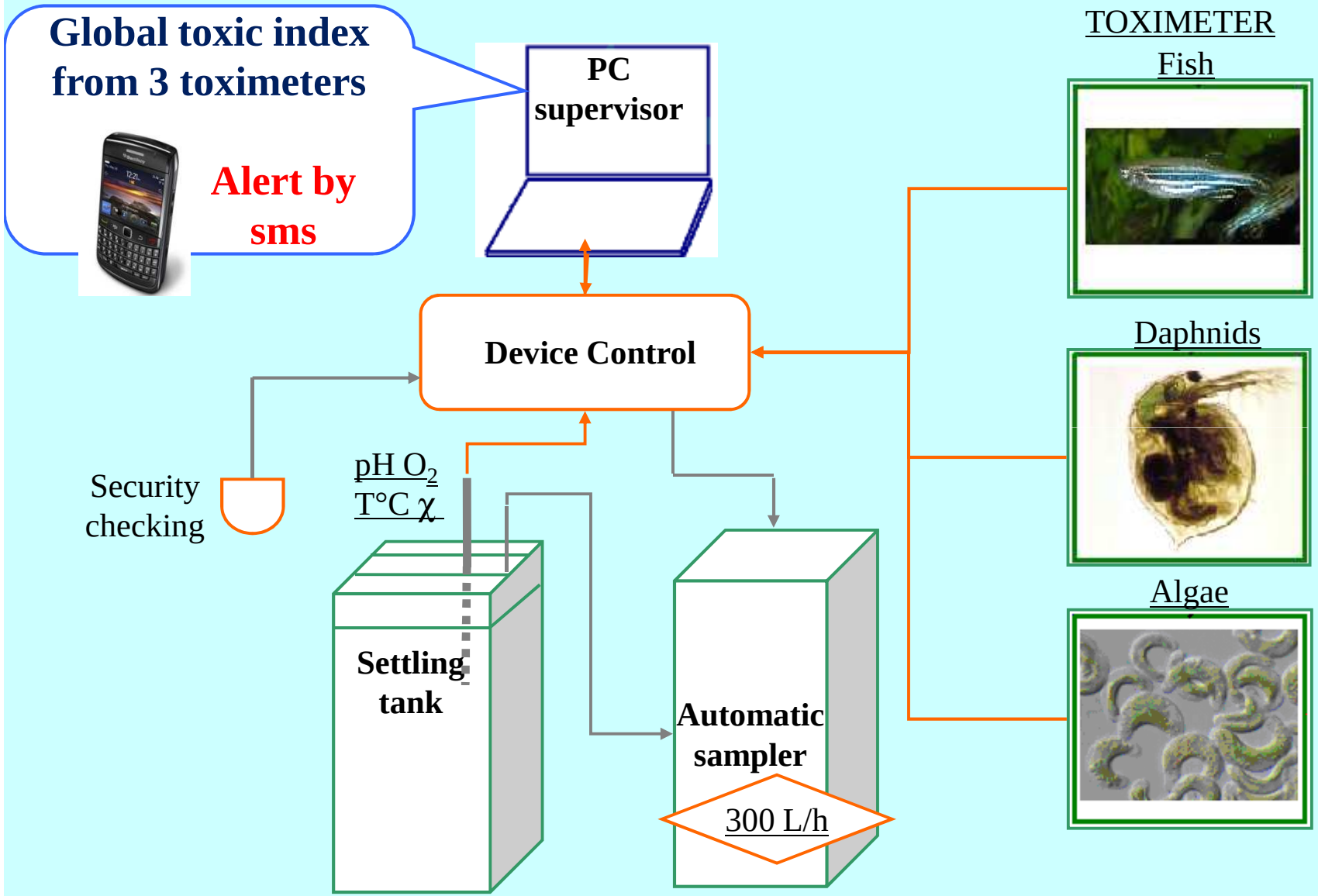
I. INTRODUCTION

Functioning of the station :

I. INTRODUCTION

Water sampling :

I. INTRODUCTION



I. INTRODUCTION

For a better understanding of behaviour endpoints :

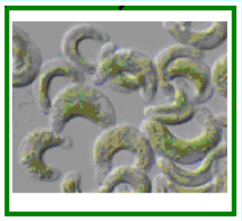
- whether compounds present in our effluents may induce alarms
- How to interpret detected alarms

Focus on the Daphnia toximeter for our research project

➢Fish toximeter

➢Daphnia toximeter

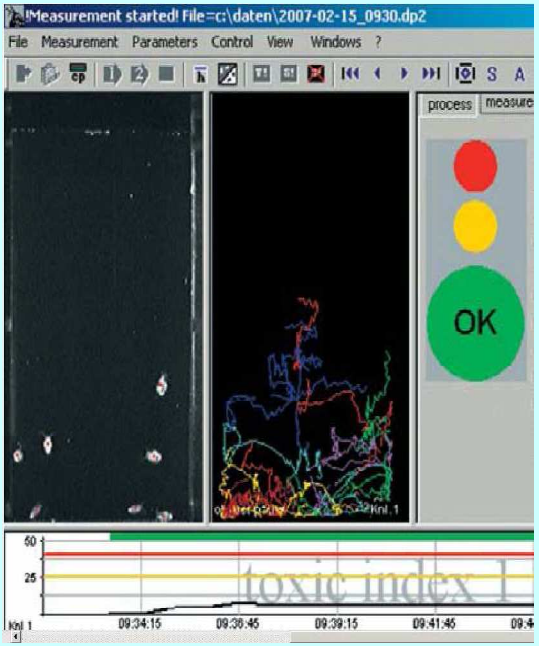
➢Algae toximeter



INTRODUCTION

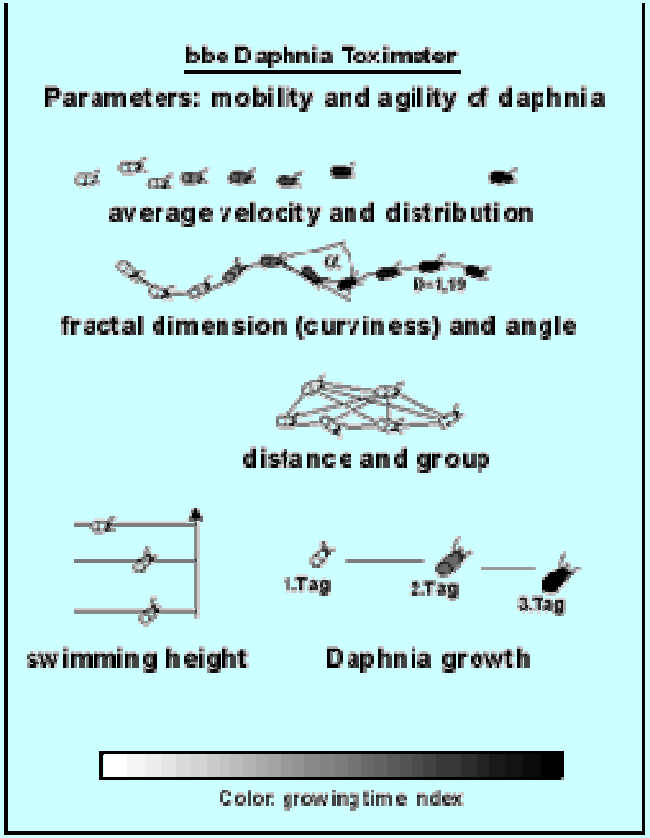
The bbe® Daphnia toximeter :

10 daphnids



INTRODUCTION

- Behavioural parameter as endpoints :



Toxicity detection with regard to the following parameter :

-average of swimming velocity

-Velocity distribution

-Average of swimming height

-Average distance between organisms

-Number of active organisms

-Average size of organisms

-fractal dimension

→ TOXIC INDEX

INTRODUCTION

Good points of the bbe Daphnia toximeter:

- fast detection (compared to 48 hours standards tests)
- continuously running during 7 days (without maintenance)
- Real time analysis, detection of short term pollution peaks
- time-dependant variations
- numerous application (Surface water quality, drinking water distribution i
  antiterrorism chemical weapons control)

→ ***This system is a good tool for early warning biomonitoring system in the field***

However, we do not really know what we are actually measuring.

INTRODUCTION

Open questions concerning Daphnia toximeter :

- How sensitive is the daphnia toximeter?
- What compounds can be detected by the daphnia toximeter?
  - At which concentrations?
  - After how long exposure times?
- Can we observe dose-dependent behavioural effect?

INTRODUCTION

Underlying scientific questions :

(i) What kind of altered behaviour can we expect? Desorientation (turn angle), narcotizing effect(resting), excitation

(ii) What compounds lead to detectable changes in *Daphnia magna* behaviour?

(iii) What is the sensitivity of behaviour endpoints versus standard tests (mortality 48h)?

(iv) How behavioural responses can be used as ecotoxicological endpoints?

⟶ ***Aim of the study: Establish an understanding of movements behaviour of the aquatic micro-invertebrate Daphnia magna***

I. Previous results on Daphnia toximeter

Data gathered from differents daphnia toximeter users :
(source: M. Lechelt)

Substance	Kind of substance (family)	EC 50	Alarm threshold	Source
Aldrin	Insecticide / Acaricide (Organochloré Highly lipophilic)	28 µg/l	> 27 µg/L	Institute for Sanitation and Environment Hamburg 2005
Carbaryl	Insecticide (Carbamate)	24h-EC50 age < 24h = 18,6 µg/L 24h-EC50 age 7-8d = 38,7 µg/L	22 µg/L	Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg (LUBW) 2006
Carbofuran	Insecticide (Carbamate)	24h-EC50 age < 24h = 80 µg/L 24h-EC50 age 7-8d = 489 µg/L	110 µg/L	Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg (LUBW) 2006
Chlorfenvinphos	Insecticide / Acaricide (organophosphoré)	30 µg/l	> 30 µg/L	Institute for Sanitation and Environment Hamburg 2005
Chlorfenvinphos	Insecticide / Acaricide (organophosphoré)	30 µg/l	> 30 µg/L	Institute for Sanitation and Environment Hamburg 2005
Chloroform	Narcotics		> 10 mg/L	Landesanstalt für Umweltschutz Karlsruhe 1999
Chlorpyrifos	Insecticide (organophosphate)		> 15 µg/L	Institute for Sanitation and Environment Hamburg 2005
Cyclosarin (GF)	Neurotoxin (organophosphate)		> 10µg/L	Wehrwissenschaftliches Institut für Schutztechnologie der Bundeswehr
Cypermethrin	Insecticide (pyréthrinoïde de synthèse)	24h-EC50 age < 24h = 1,17 µg/L 24h-EC50 age 7-8d = 15,4 µg/L	1 µg/L	Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg (LUBW) 2006
Diazinon	Insecticide (organophosphate)		> 100 µg/L	Rhine Water Control Station Worms 9/2003
Dichlorvos	Insecticide (organophosphate)	170 µg/l	> 0,5 - 1 µg/L	Institute for Sanitation and Environment Hamburg 2005
Dimethoat	Insecticide (organophosphorés)	24h-EC50 age < 24h = 1,9 mg/L 24h-EC50 age 7-8d = 1,85 mg/l	2100 µg/L	Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg (LUBW) 2006
Endosulfan	Insecticide / Acaricide (organoclhorés)	0,2-0,9 mg/l	> 100 µg/L	Landesanstalt für Umweltschutz Karlsruhe 1999
Endosulfan	Insecticide / Acaricide	0,2-0,9 mg/l	> 500 µg/L	Institute for Sanitation and Environment Hamburg 2005
Endosulfan	Insecticide / Acaricide	0,2-0,9 mg/l	> 200 µg/L	Institute for Sanitation and Environment Hamburg 2005
Esfenvalerate	Insecticide (pyrétrhrinoïde de synthèse)		> 1 µg/L	Institute for Sanitation and Environment Hamburg 11/2004
Hexachlorcyclohexan (α - HCH) (lindane)	Insecticide (halocarbures)	0,2 - 1,7 mg/l	> 1000 µg/L	Institute for Sanitation and Environment Hamburg 2005
Hexachlorcyclohexan (β - HCH)	Insecticide (halocarbures)		> 200 µg/L	Institute for Sanitation and Environment Hamburg 2005
Hexachlorcyclohexan (δ - HCH)	Insecticide (halocarbures)		> 100 µg/L	Institute for Sanitation and Environment Hamburg 2005
l-Cyhalothrin	Insecticide (pyréthrinoïde de synthèse)		> 500 µg/L	Umweltbehörde Hamburg 8/2000
Lindane	Insecticide	24h-EC50 age < 24h = 1,03 mg/L 48h-EC50 age 7-8d = 2,54 mg/l	650 µg/L	Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg (LUBW) 2006
Lindane	Insecticide	0,8 - 6,5 mg/l	> 30 µg/L	Landesanstalt für Umweltschutz Karlsruhe 1999
Lindane (γ - HCH)	Insecticide (organochlorés)	0,8 - 6,5 mg/l	> 350 µg/L	Institute for Sanitation and Environment Hamburg 2005

- 38 alarm thresholds (24 compounds)
- 6 differents institutes
- Only insecticides / Neurotoxics

I. Previous results on Daphnia toximeter

Actual state of knowledge :

- Numerous tested substances however few modes action are represented among them

- Heterogeneity in :
  - Exposure conditions (water quality)
  - Exposure times
  - Alarm parameter settings (depending of the river characteristics)

- Homogeinety in tested compounds :
  - 80% of insecticides, 16 % of neurotoxics, 4 % energitic disturbing compound

I. Previous results on Daphnia toximeter

→ Non-conclusive results in regards to risk assessment :

- high variability in results
- no controls
- no replicates
- no concentration-response relationships

We need supplementary tests with more substances with differents mode of action (metals, oxydants…) at several concentrations and replicates

I. Previous results on Daphnia toximeter

Several substances tested by INERIS for EDF:

- Analyses are more qualitatives than quantitatives

- We can not conclude or make comparison between these tests.

I. Previous results on Daphnia toximeter

Tests of 3 differents conc. of sucralose + control on Daphnia toximeter

(A.-K.E Wiklund, 2012)

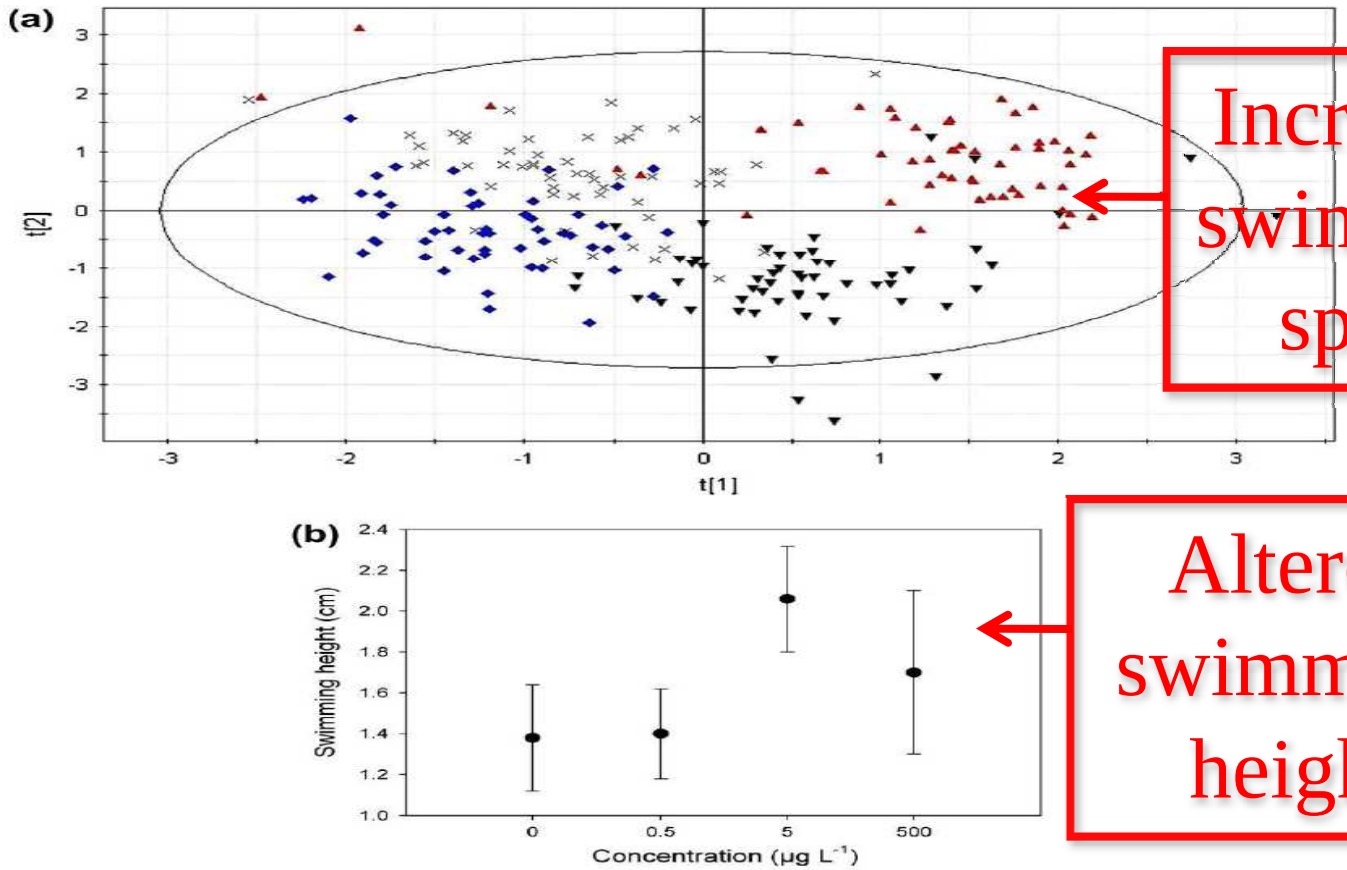


Fig. 2. (a and b) *Daphnia magna* was exposed in a Daphnia Toximeter (bbe Moldaenke, Kiel, Germany) to three concentrations of sucralose and a control. a – (top) PLS analysis of the data produced by the Toximeter. In the PLS plot ♦ = control, x = 0.5 µg L^{-1}, ▼ = 5 µg L^{-1}, ▲ = 500 µg L^{-1}. b – (bottom) Swimming height data obtained from the Toximeter. Swimming height and swimming speed (not shown) were the two factors that had the largest influence on the PLS analyses.

I. Previous results

Daphnia toximeter tests by Lewandowska, 2004:

Substance	Mode of action	EC50 (48h)	Alarm treshold (48h)
Ensfevalerate (pyrethroid)	voltage-dependent sodium-channel agonist	0.9 µg/L	1 µg/L
Trichlorfon (organophosphate)	AChE inhibitor	2 µg/L	1 µg/L

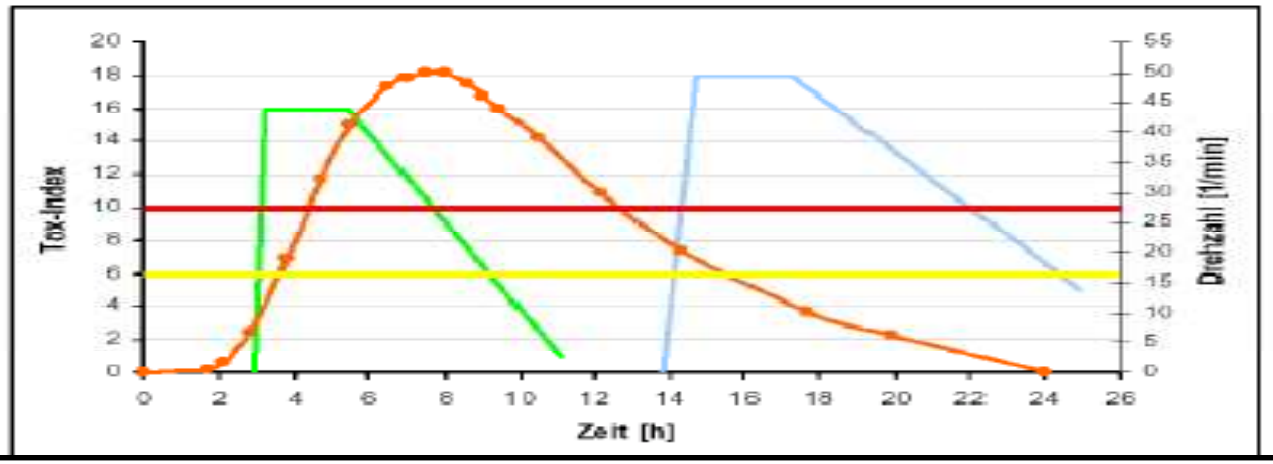
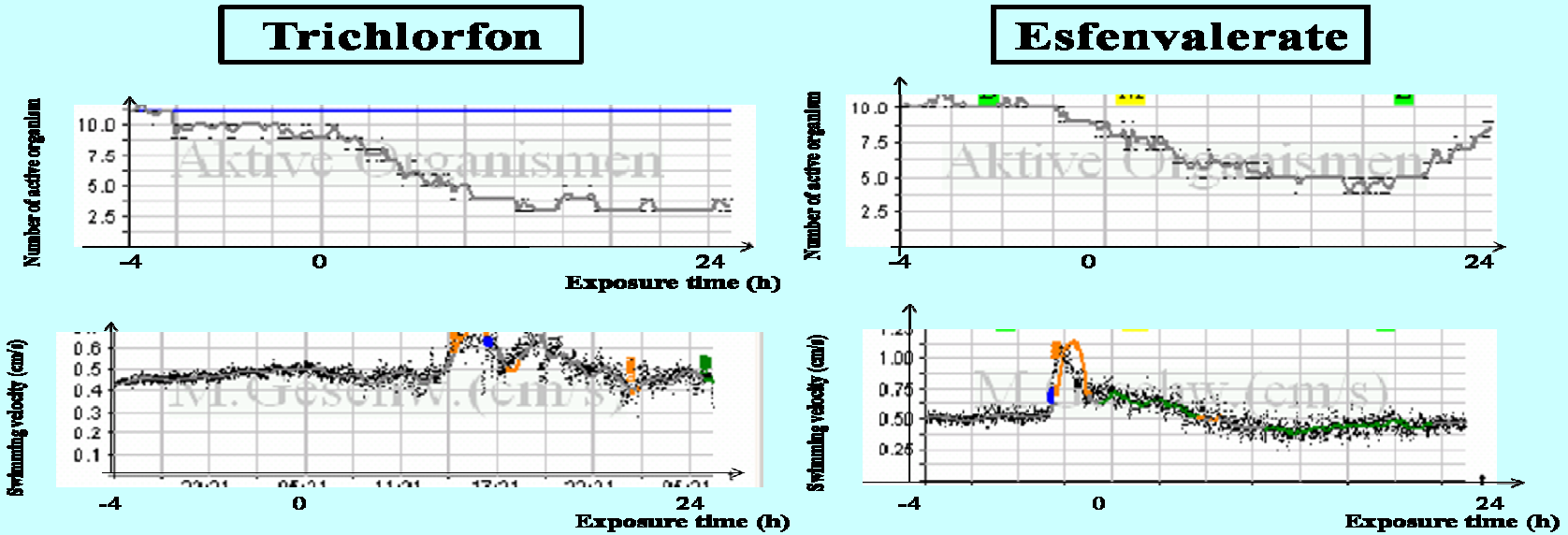


Figure 1 : Response in function of time observed after exposition at 2µg/L of two insecticides during 24 H (Source: Lewandowska, 2004).

I. Previous results

Daphnia toximeter tests by Lewandowska, 2004:



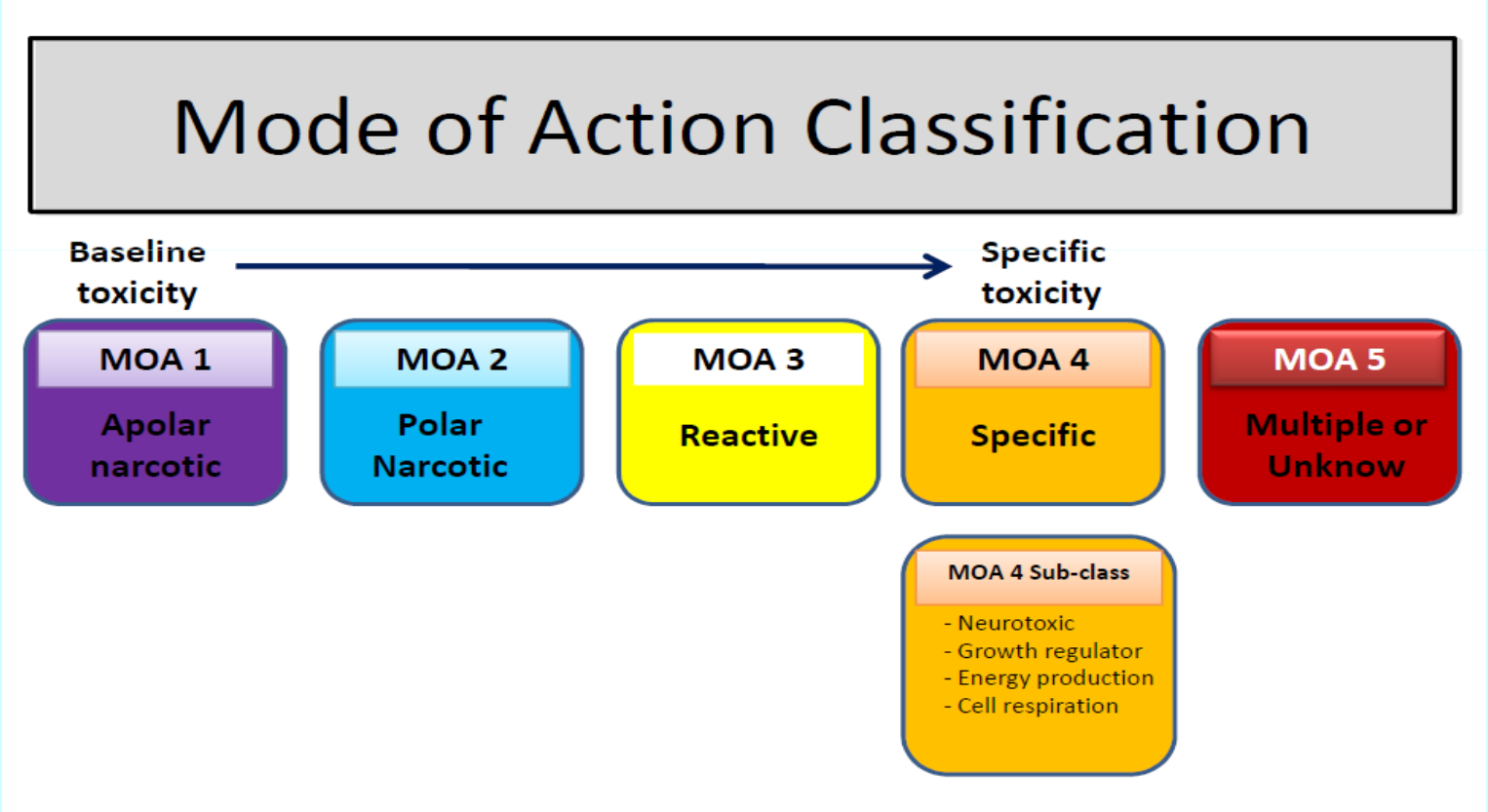
Responses observed on toximeter after an exposition of 2µg/L of two different insecticides during 24H (Source: Lewandowska, 2004)

- Latent effect of Trichlorfon on Daphnia behaviour compared to Esfenvalerate

➡ ***Hypothesis: differences in patterns are due to the specific mode action of each substance***

II. Our experimental Approach

→ What are the differents modes of action which are relevant for *D. magna*?



II. Our experimental Approach

MATERIALS AND METHODS :

Testing substances selection

The mode of action may help in:

- understanding the alteration of the behaviour in *Daphnia magna*
- Predict the time on onset effect
- Etablish a generalisation of sensitivity

Experimental protocol

- different modes of action tested under controlled conditions at several concentrations and replicates

- different parameters monitored during the whole exposure time of 48 hours:
  - Number of active organisms
  - Individual swimming velocity average (1 min.)
  - Angular velocity

III. Our experimental Approach

Conception of a new system with Viewpoint®

9 daphnids/ chamber

24 optic glasses as exposure chambers

Dark box (100 x 60 x 60 cm) for observation in complete darkness

Infra-red sensitive camera

Red or Infra-Red LED

Hygrometric system

Risk Assessment → **Biomonitoring**

Standards test OCDE (48H)		VIEWPOINT® SYSTEM		Daphnia toximeter
• Static	=	• Static	≠	• Continuous flow
• 48 H	=	• 48 H	≠	• 7 days
• No acclimation (exposure at 0 to 48h)	=	• No acclimation (exposure at 0 to 48h)	≠	• Acclimation (before exposure)
• Replicates	=	• Replicates	≠	• 1 or 2 measuring cells
• Results at 0 and 48 h	≠	• recording raw data	=	• recording raw data
• No software	≠	• Zebralab software	≃	• bbe software

Conclusion and Perspectives

- Consequences of Behavioural effects must be condidered seriously !
  - It cause the animal to diverge from normal behaviour
  - It may have significant ecological consequences (on reproduction or prey/predator relationship)

- Actual results do not allow to conclude about the sensitivity of behaviour parameter

- We develop a new system which allow to make tests of different substances at differents concentrations and replicates.

→ ***Perspectives: We hope to better understand the behaviour for describe quantitatively the responses***

Thank you for your attention