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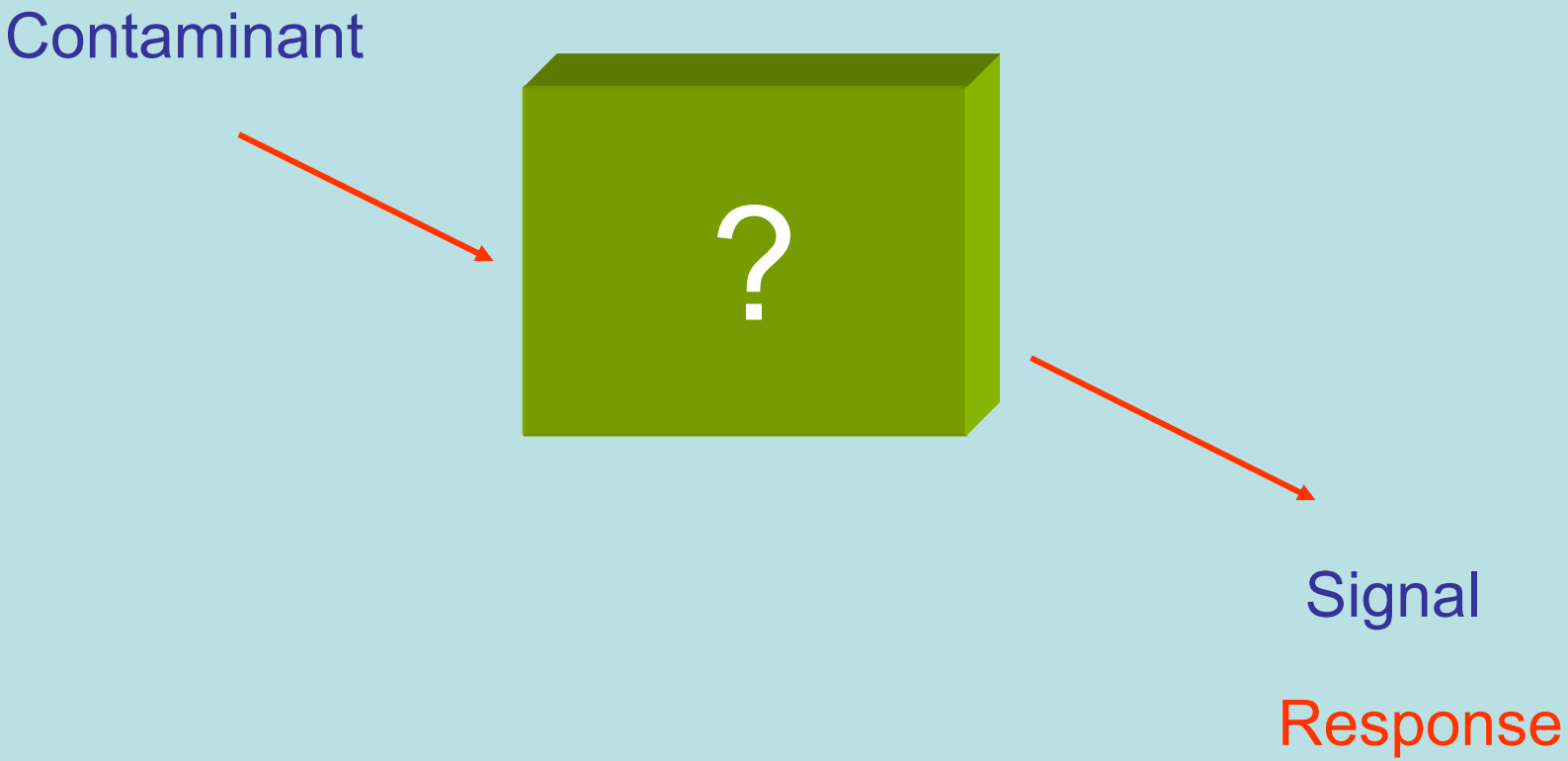
Metodi funzionali nella rilevazione di biotossine algali: criticità e opportunità

Regulation 853/2004/EC establishes the maximum levels of contaminations for some marine biotoxins in shellfish.

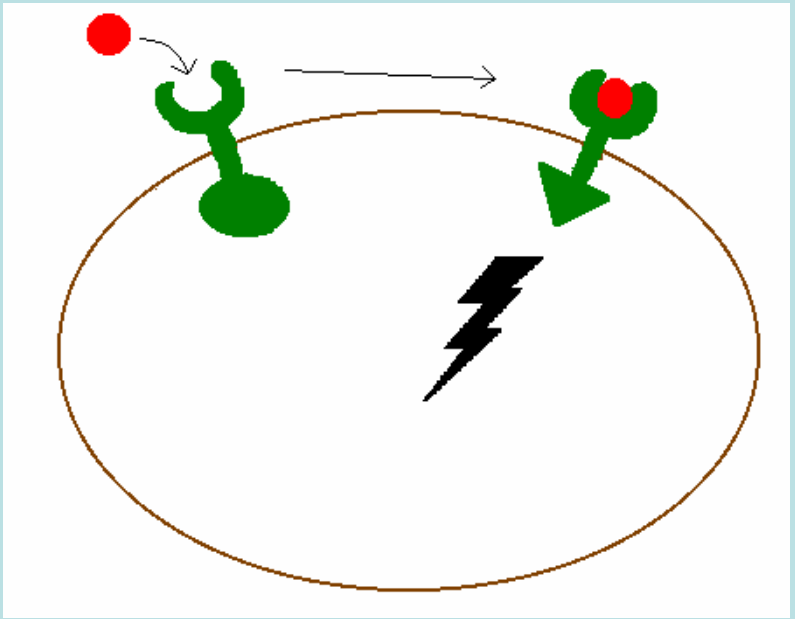
Regulation 2074/2005/EC lays down the recognised testing methods for detecting marine biotoxins .

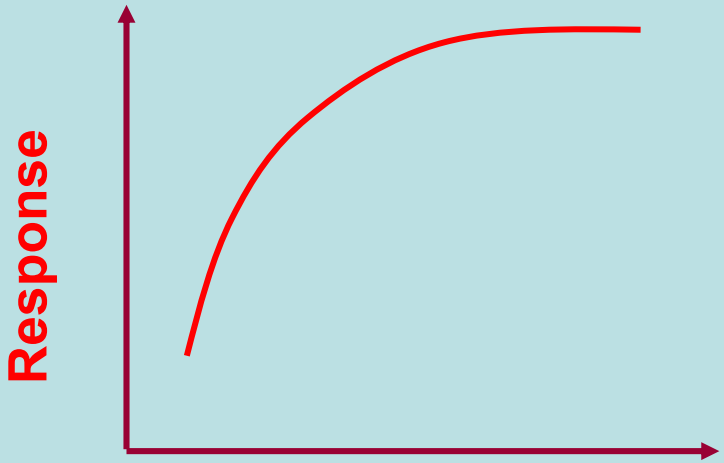
but

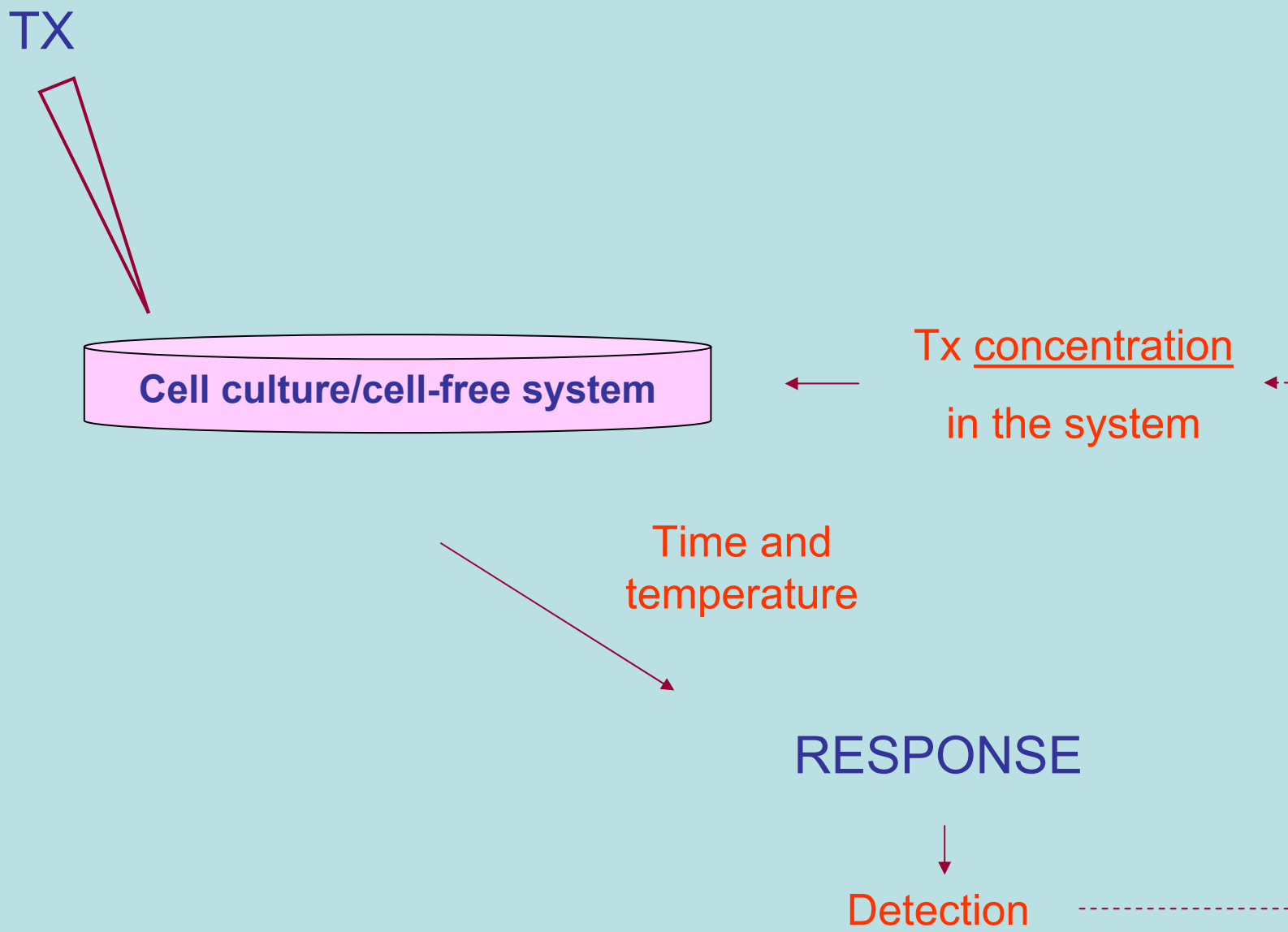
Directive 86/609/EEC makes provision for laws, regulations and administrative provisions for the protection of animals used for experimental and other scientific purposes



Functional assays in marine biotoxin detection
(Rossini: *Toxicology*; **208**, 2005, 451-462)




Response
TX concentration


TX
Cell culture/cell-free system
Tx concentration
in the system
Time and
temperature
RESPONSE
Detection

• In functional assays the ***measurand*** is a biological response integrating the overall levels of biologically active compounds, acting through the same receptorial component.

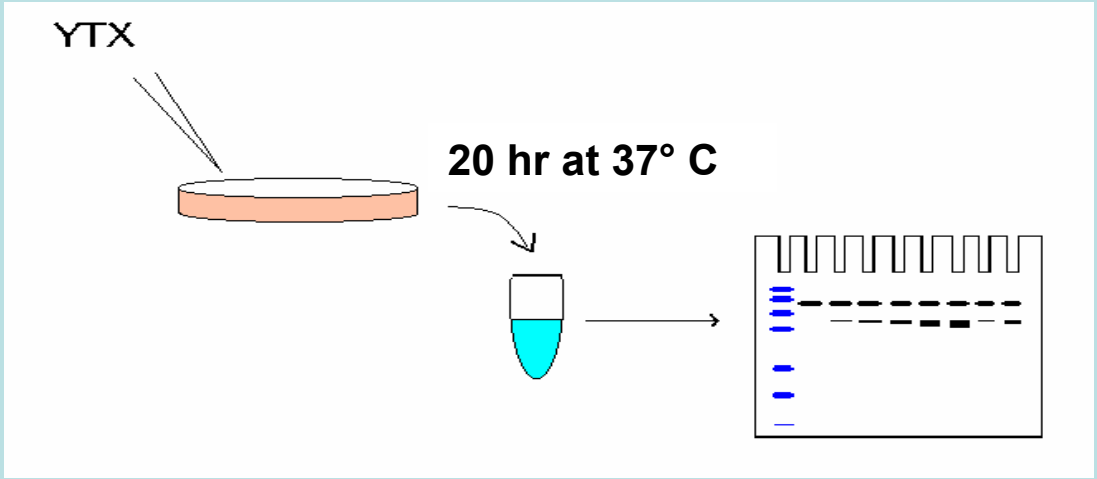
• The **quantity** of biologically active compounds is determined by comparing the effects induced by an unknown and by a reference compound.

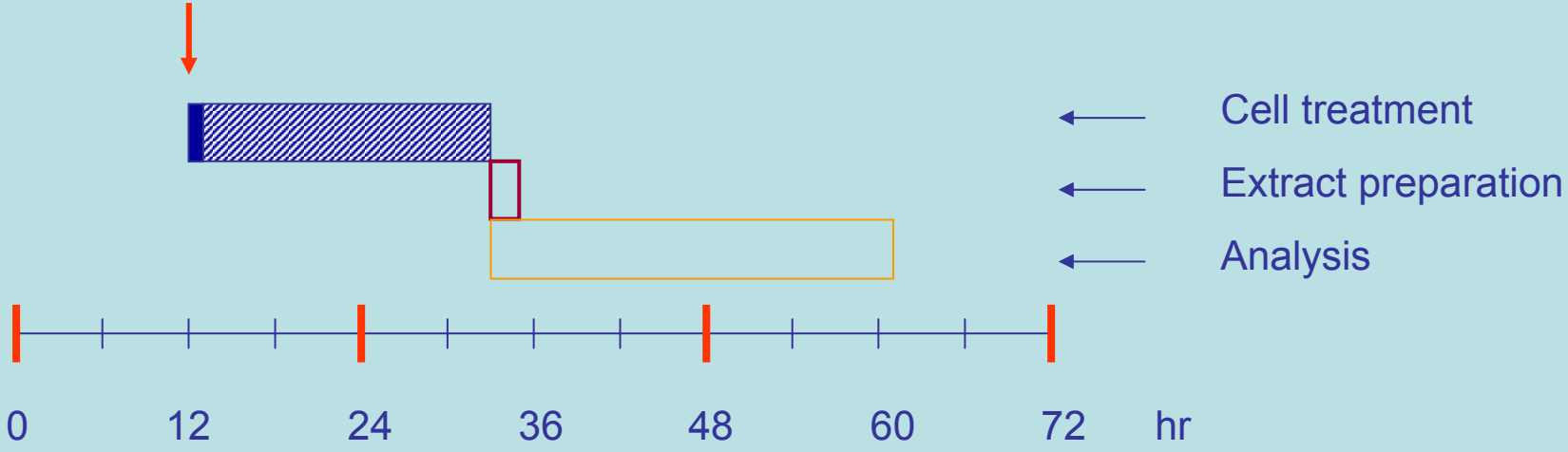
• The estimates are expressed in **Toxin Equivalents**

The relationship between the concentration of the isolated ***analyte*** and the ***signal*** of the measuring ***instrument***

The *instrument* is a biological system

The *signal* is the system's response, that an operator will detect by the use of additional procedures



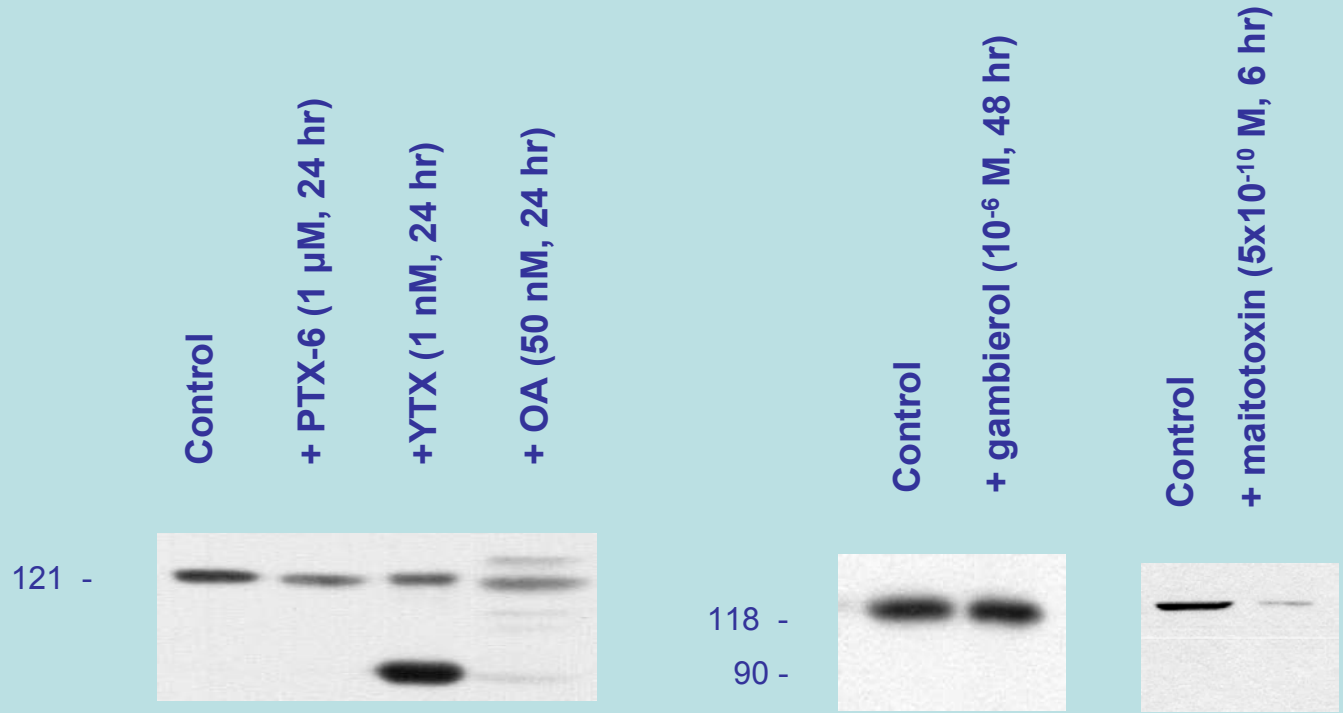


30 major steps (each composed of m, n,….z operations)

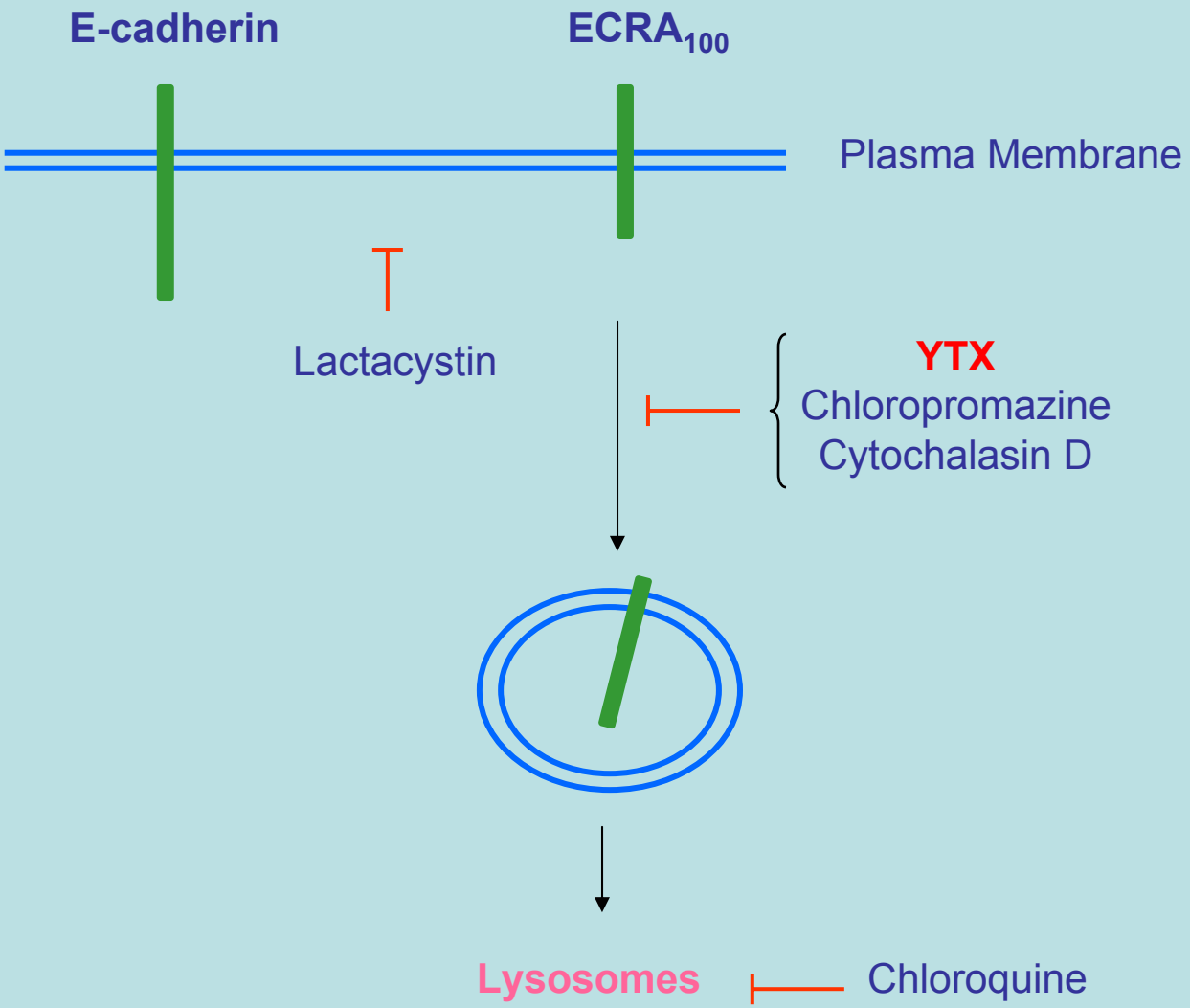
Functional assay to measure yessotoxins in contaminated mussel samples (Pierotti et al.: *Anal. Biochem.*; **312**, 2003, 208-216)

Familiarization workshop in MO: YTX levels in one material (four "new" operators from different institutions, same equipment)

Participant	YTX content (µgE YTX/kg)	Intra-assay (%)	Mean ± SD	Inter-assay (%)	Expected	Var (%)
1	823 ± 197	18.1	774 ± 135	12.6	741	4.4
2	753 ± 124	11.8				
3	600 ± 115	14.0				
4	920 ± 145	11.5				



Azaspiracid-1 alters the E-cadherin pool in epithelial cells (Ronzitti et al.; *Toxicol. Sci.*; **95**, 2007, 427-435)



Yessotoxin inhibits the complete degradation of E-cadherin
(Callegari & Rossini; *Toxicology*; **244**, 2008, 133-144)

Critical points for the implementation of functional assays

Complexity of the assay/procedure

Specificity and general performance (fit for purpose)

Dissemination of the methodology

Standardization and validation of procedures

Acceptance

Opportunities of functional assays

Toxicity Testing in the 21st Century

A vision and a strategy

The US National Research Council of the National Academy of Sciences

2007

Current – studies that evaluate observable outcomes in whole animals, which generate data on apical end points

Critical points

- **Coverage of a limited number of chemicals**
- **Costly and time-consuming**
- **Too many animals used in testing**
- **Limited scientific (mechanistic) bases for assessing health effects**

Toxicity Testing in the 21st Century

A vision and a strategy

The US National Research Council of the National Academy of Sciences

Paradigm shift – From the current apical end point based system to a mechanistically based test system, integrating

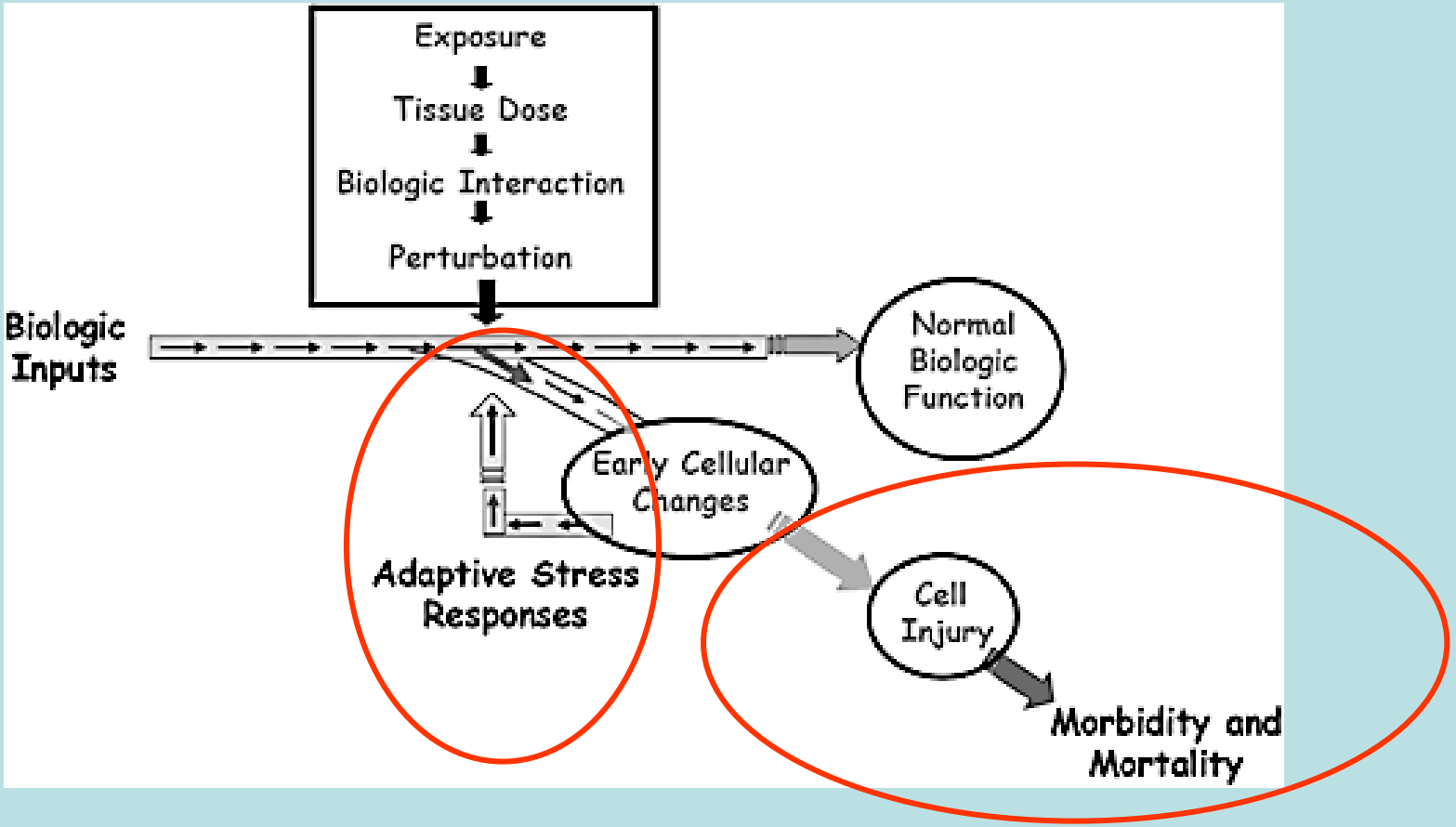
- ***in vitro* tests that assess critical mechanistic end points**
- **targeted *in vivo* tests that ensure adequate testing of metabolites and coverage of end points**

In vitro tests

- **conducted with cells, optimally with human cells**
- **based on characterization of *toxicity pathways***

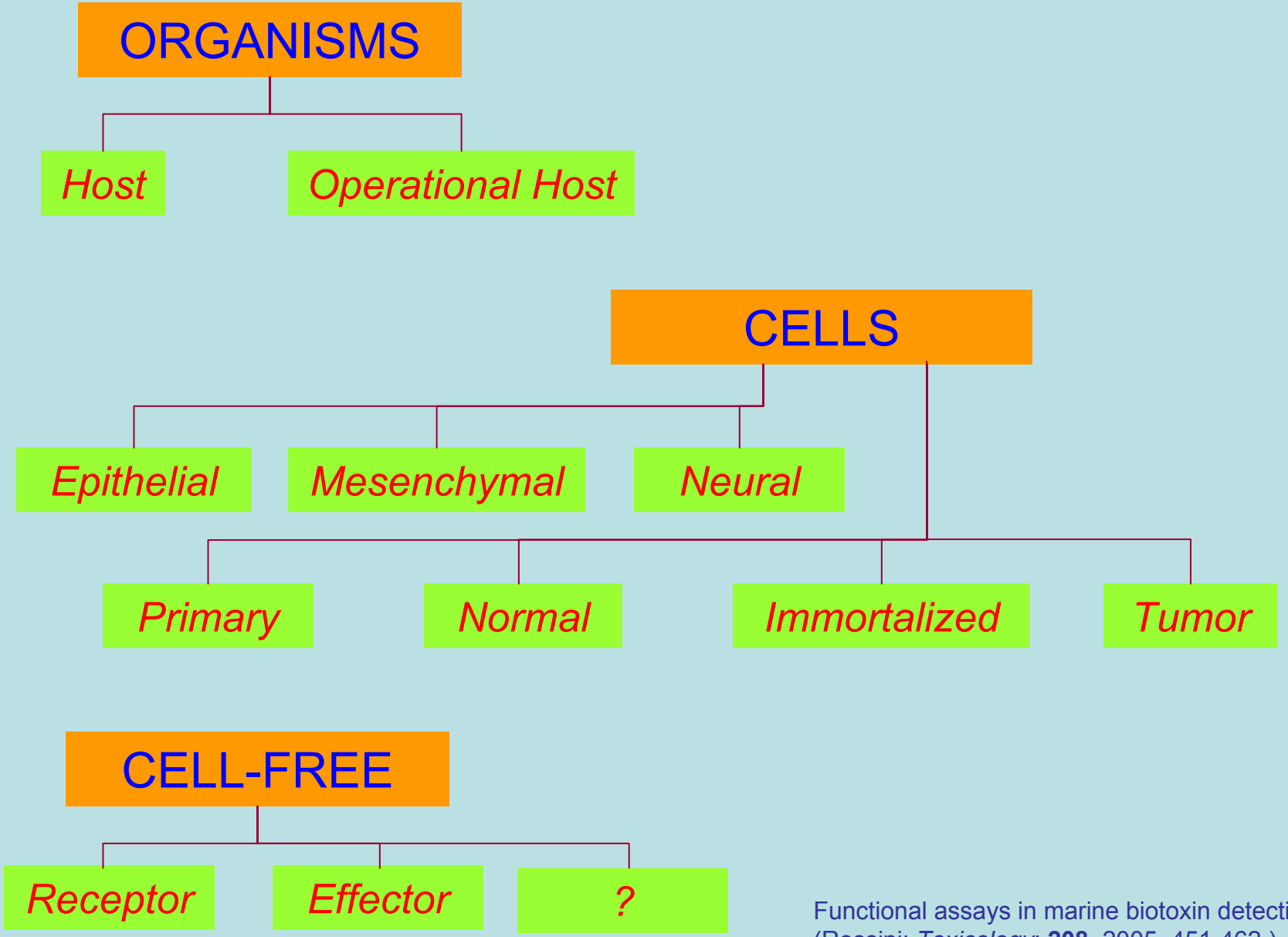
Toxicity pathways

Normal cellular response pathways that are expected to result in adverse health effects when sufficiently perturbed



Challenges of the mechanism-oriented testing paradigm

- availability of a suites of *in vitro* tests
- availability of targeted *in vivo* tests
- models of toxicity pathways
- infrastructure changes
- valdation of tests and test strategies
- acceptance of the idea that the results of tests based on perturbation of toxicity pathways are adequately predictive of adverse responses and can be used in decision-making



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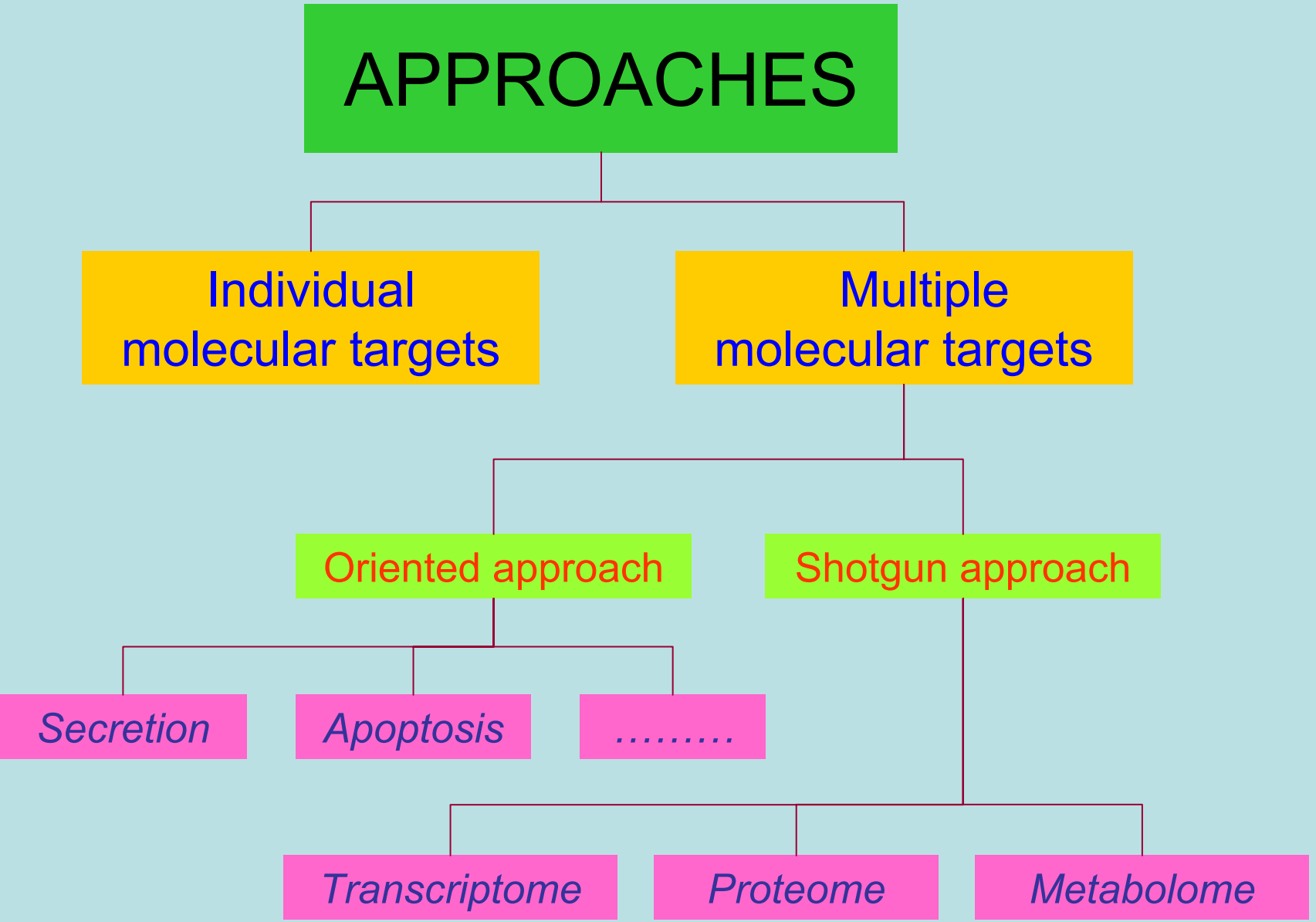

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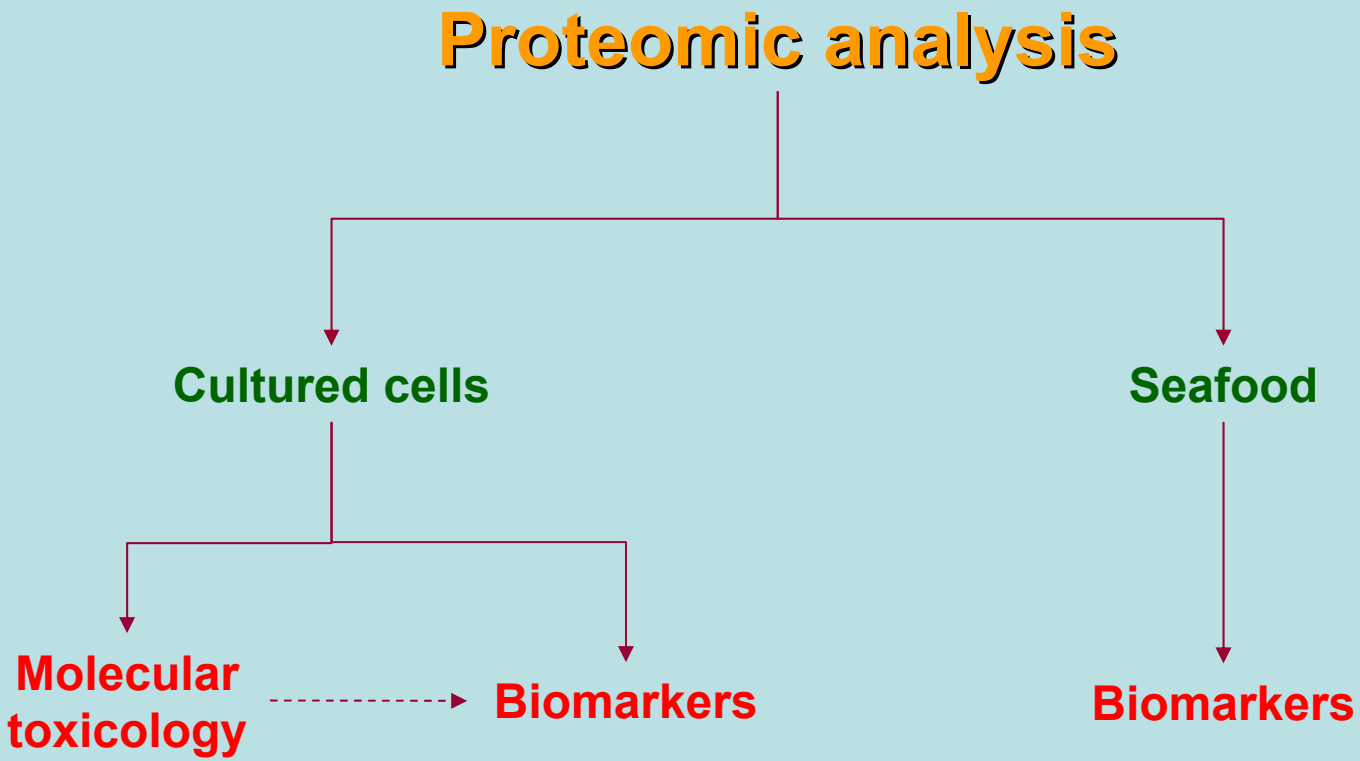
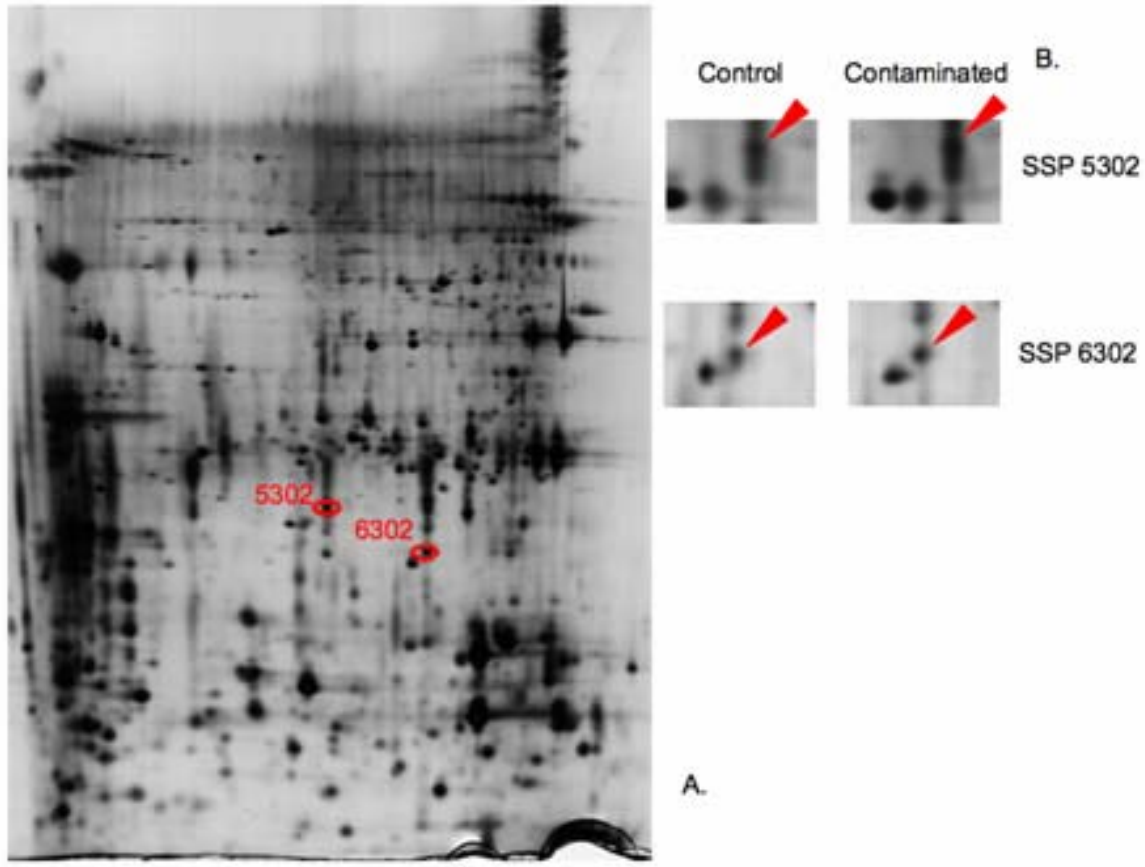

Proteomic analysis
Cultured cells
Seafood
Molecular toxicology
Biomarkers
Biomarkers

Impiego dell'analisi proteomica nella rilevazione della contaminazione di mitili da parte di biotossine

- **Acido okadaico e yessotossine**
- **Analisi appaiata di estratti da ghiandole digestive con metodi LC-MS e protein profiling**

Protein markers of algal toxin contamination in shellfish (Ronzitti et al.,: *Toxicon*; 5**2**, 2008, 705-713)



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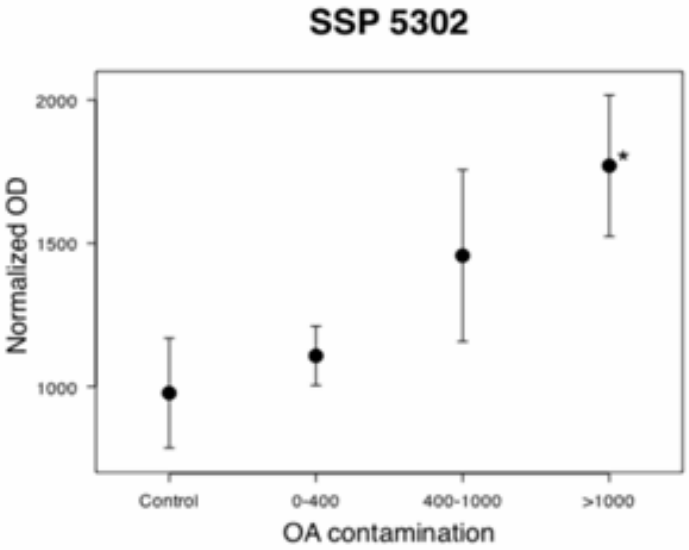

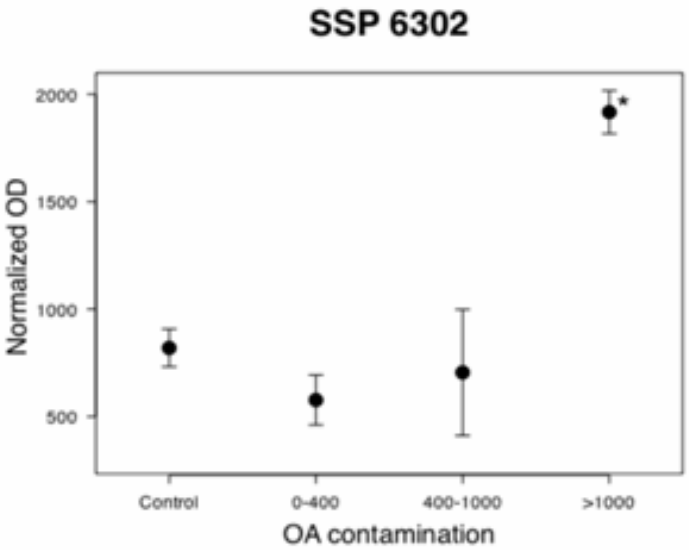

Protein markers of algal toxin contamination in shellfish (Ronzitti et al.,: *Toxicon*; 5**2**, 2008, 705-713)

Component	SSP 5302	SSP 6302
Putative identification	Photosystem Q(B)	Unknown
Peptide	R.EWEFSYR.L	R.LSMQVKSAAR.V
Peptide homology in *Mytilus*	none	**NADH dehydrogenase**
Peptide homology in other species	***Dinophysis* spp.** (DTX producers)	*n.a.*

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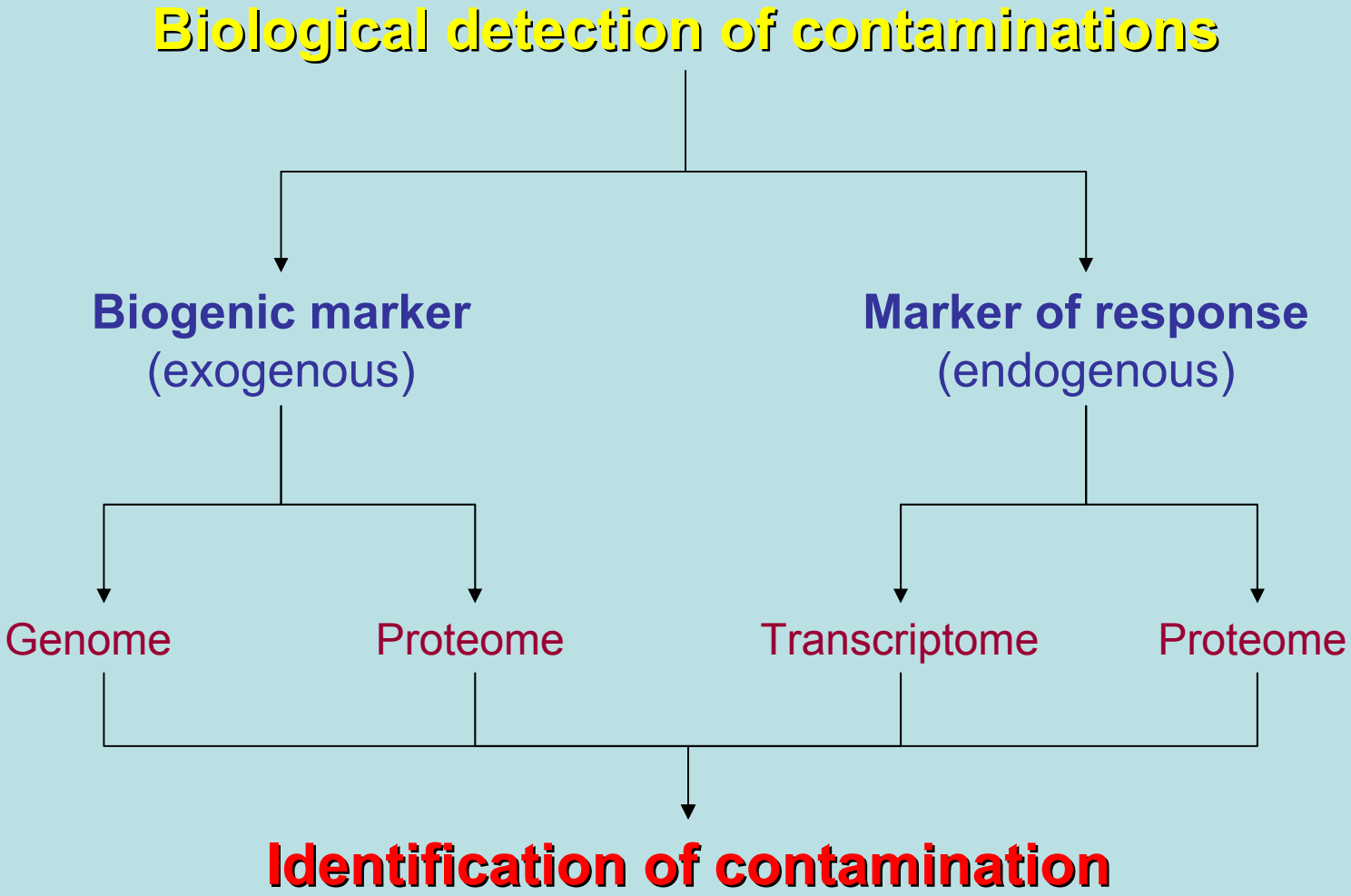
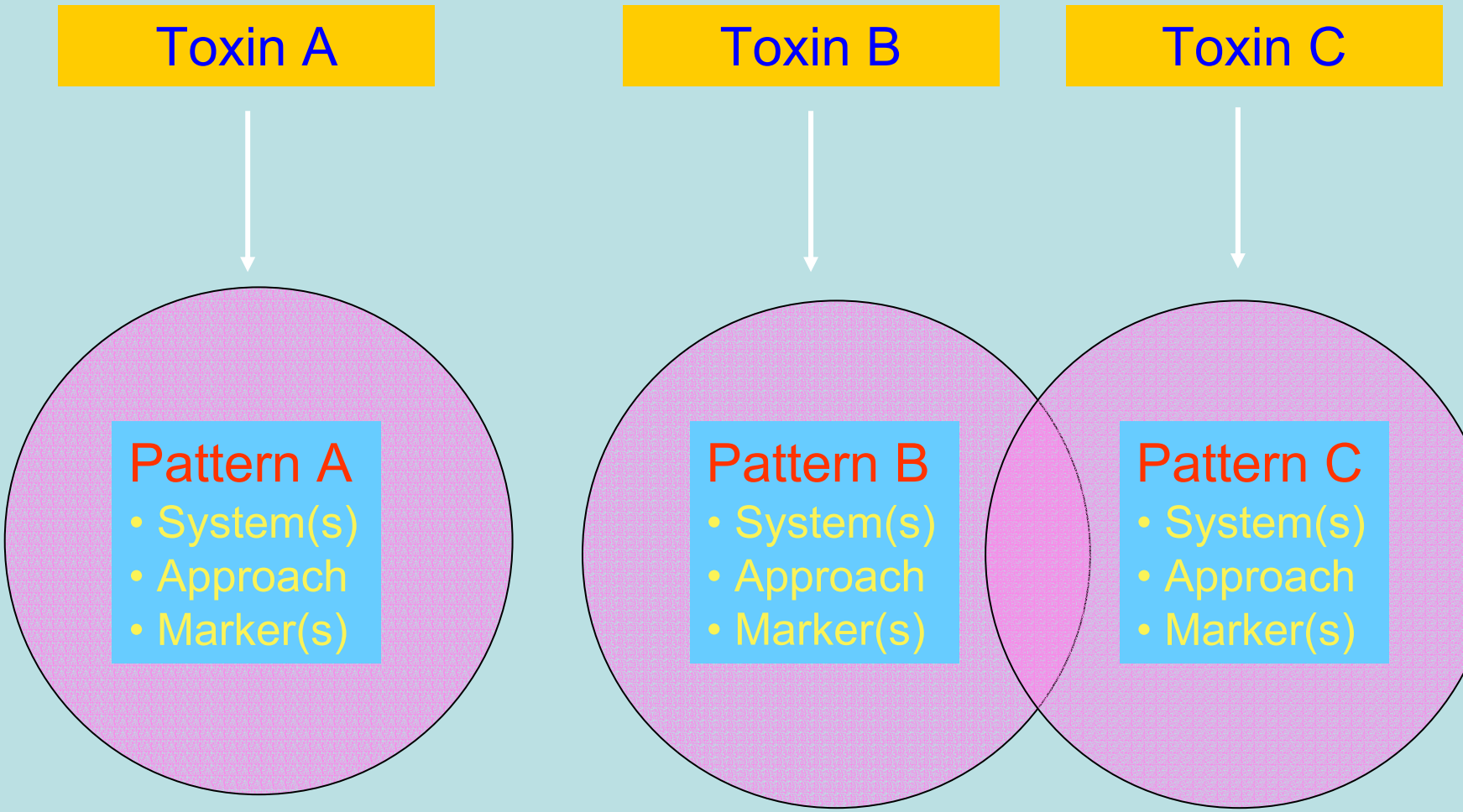
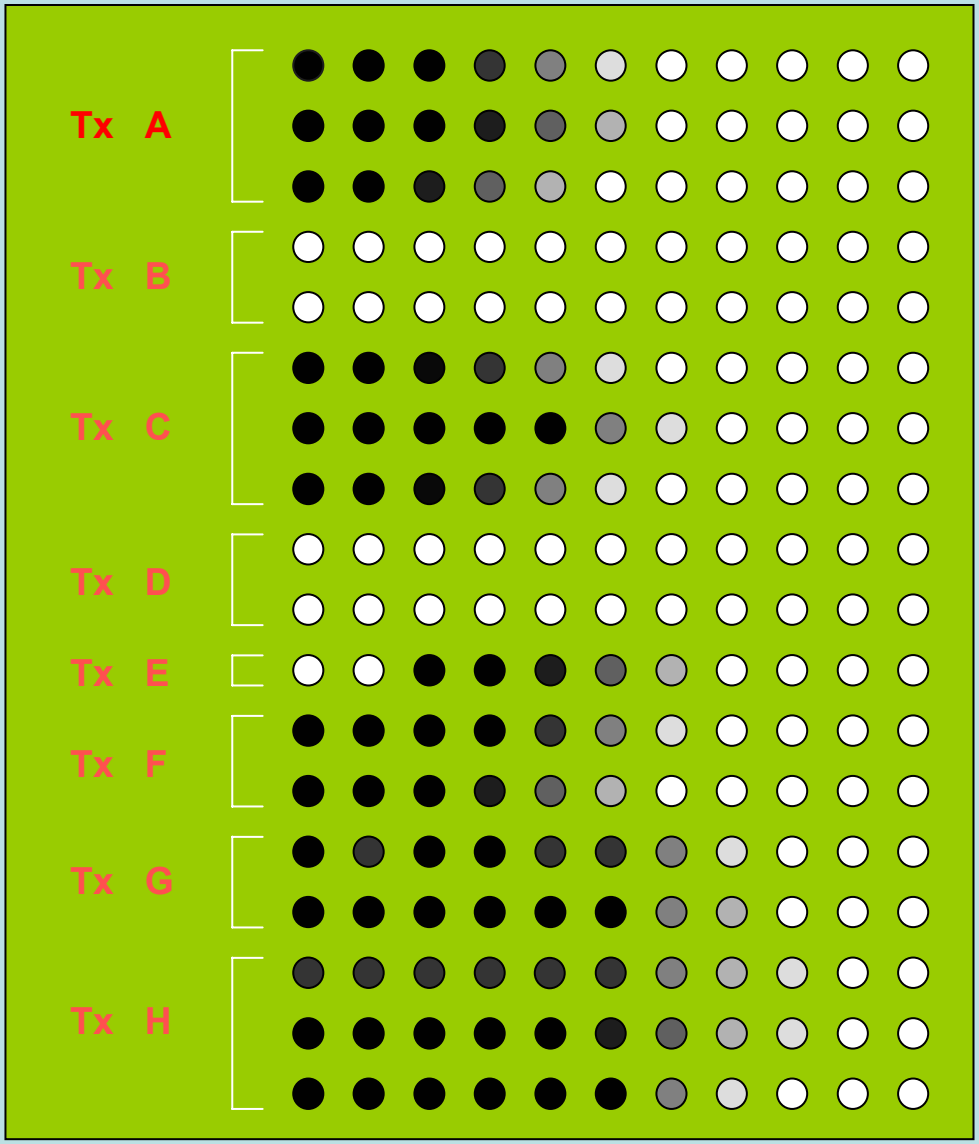

Biological detection of contaminations
Biogenic marker
(exogenous)
Marker of response
(endogenous)
Genome
Proteome
Transcriptome
Proteome
Identification of contamination


Toxin A
Toxin B
Toxin C
Pattern A
• System(s)
• Approach
• Marker(s)
Pattern B
• System(s)
• Approach
• Marker(s)
Pattern C
• System(s)
• Approach
• Marker(s)


Tx A
Tx B
Tx C
Tx D
Tx E
Tx F
Tx G
Tx H