

Ecotoxicogenomics, understanding the underlying stress mechanisms

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Outline

- ☐ **Ecotoxicogenomics: concepts and context**
- ☐ **Transcriptomic tools**
- ☐ **Tools developed in house**
- ☐ **Available genomic tools in soil ecotox**
- ☐ **Methodology**
- ☐ **Case study**
- ☐ **Output**



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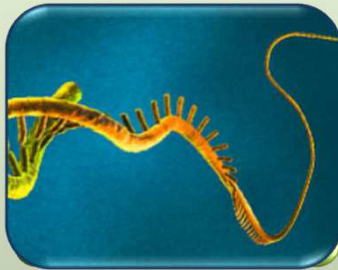


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Ecotoxicogenomics

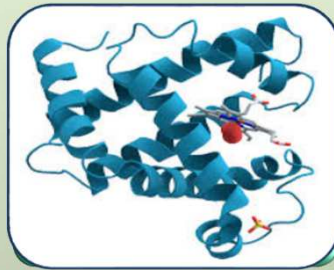
GenOMICS → Ecotoxicology

TranscriptOMICS



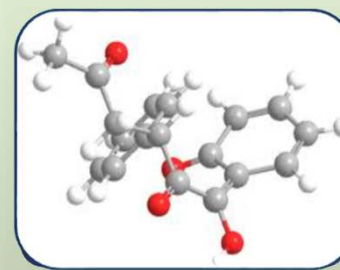
mRNA

ProteOMICS



proteins

MetabolOMICS



metabolites



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Mechanistic value



Ecological value



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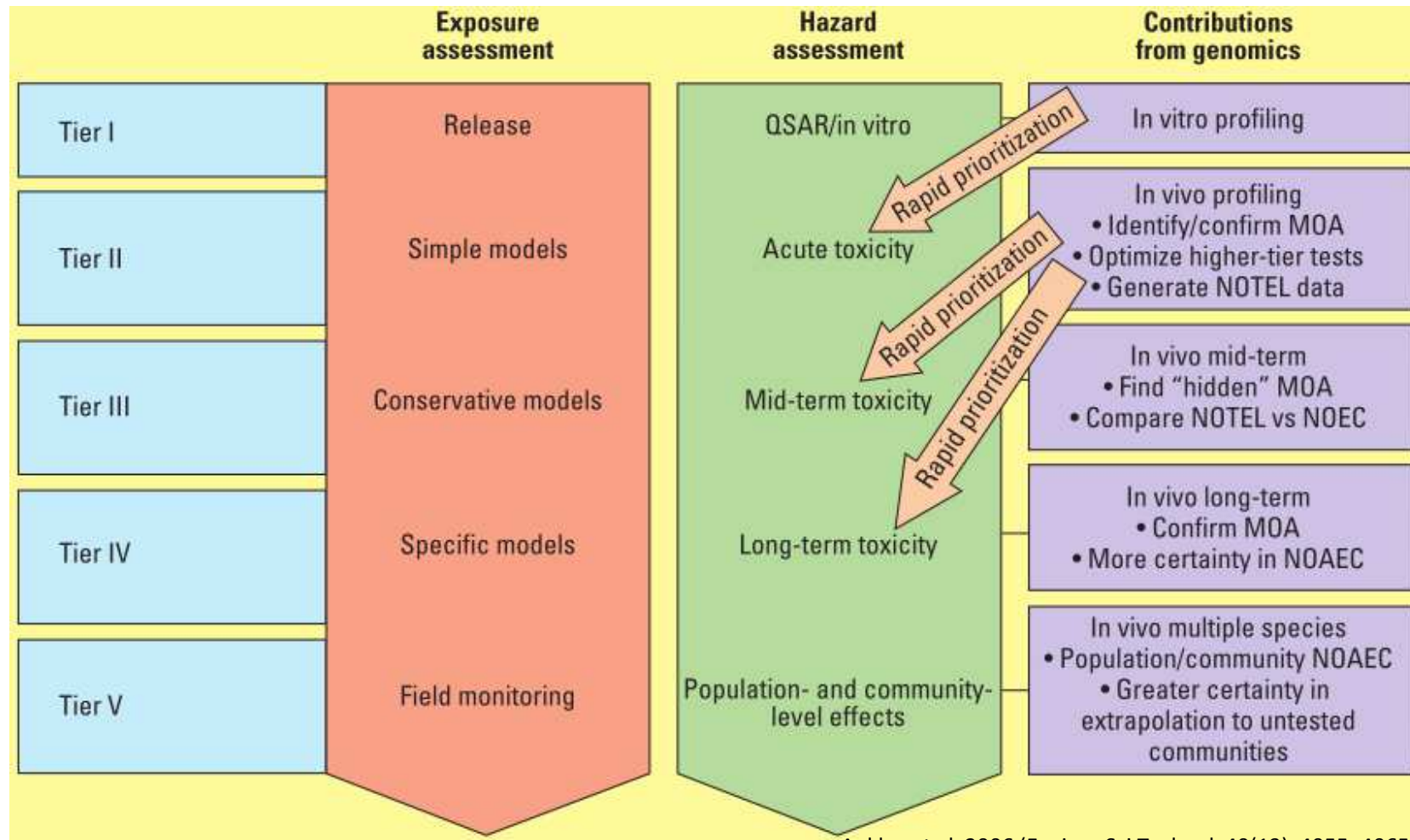
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Ecotoxicogenomics

... benefits for regulatory ecotoxicology



Ankley et al. 2006 (Environ Sci Technol. 40(13): 4055–4065)



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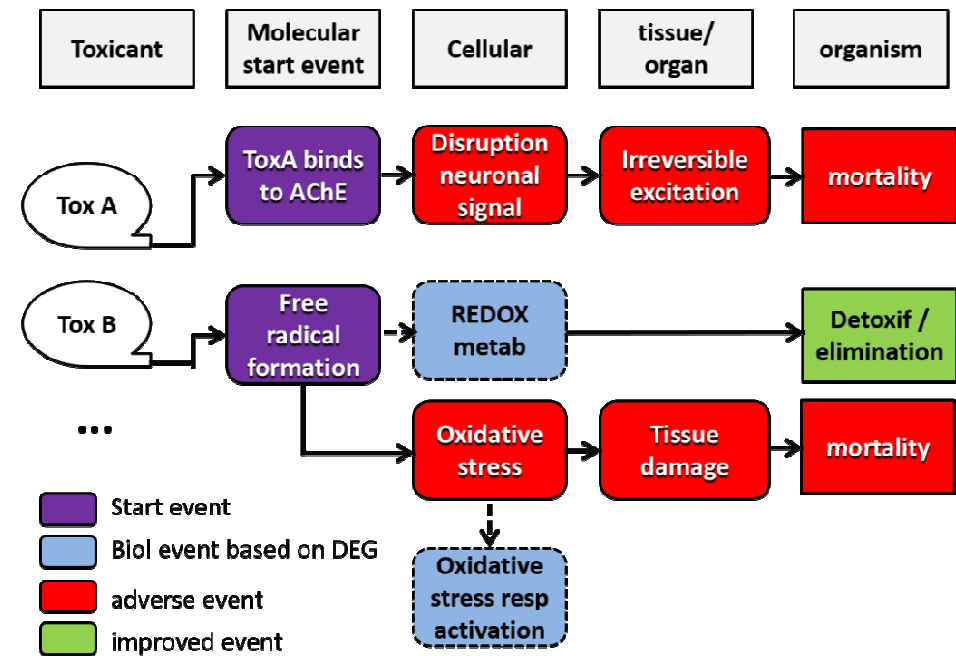


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Context

➤ Effects at various levels:

- Population
- Organism
- Cell
- Sub-cellular



➤ Integration: SYSTEMS TOXICOLOGY

➤ AOP – Adverse Outcome Pathways



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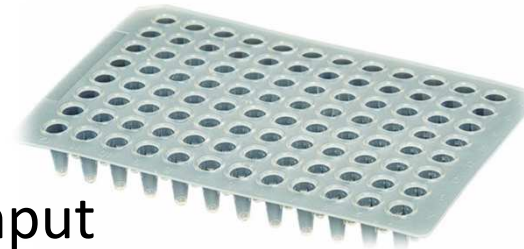
(transcriptomic) Tools

qPCR

Quantitative gene expression

Limited no of genes – low-throughput

Limited to previous knowledge/probe design

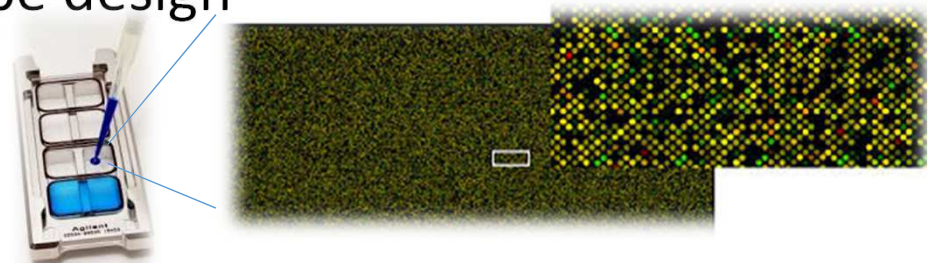


(DNA) μ arrays

Semi-quantitative gene expression

Can be whole transcriptome and is EST

Thousands of genes in one run – high-throughput (HTP)



RNA-seq

Can be quantitative, still very expensive for that purpose

Whole transcriptome in real time/HTP (de novo assembly) – Not EST

Not limited to previous knowledge/probe design



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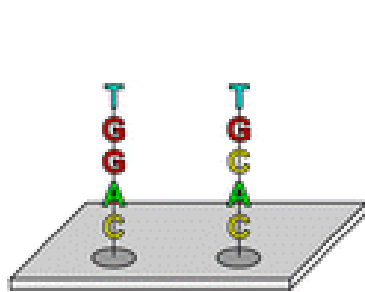
(transcriptomic) Tools

(DNA) μ arrays

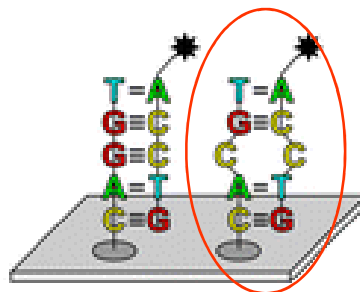
Perforated solid surface (glass) containing a small ssDNA sequence (probe) in each spot

Principle of the method:

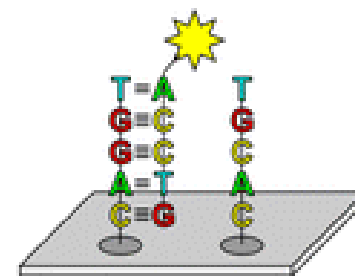
Quantification (fluorescence based) of the specific probe-target hybridisation



Probes
on the microarray



Unspecific hybridisation
removed by wash



Fluorescence detection
of the hybrid

Adapted from: Seela and Budow 2008 (Mol. BioSyst., 4, 232-245)



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Tools: development of μ array

SETAC PRESS

1

Environmental Toxicology and Chemistry, Vol. 30, No. 6, pp. 1395–1402, 2011
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Printed in the USA
DOI: 10.1002/etc.512

DEVELOPMENT OF A MICROARRAY FOR *ENCHYTRAEUS ALBIDUS* (OLIGOCHAETA): PRELIMINARY TOOL WITH DIVERSE APPLICATIONS

MÓNICA J.B. AMORIM,*† SARA C. NOVAIS,† KARLIJN VAN DER VEN,‡ TINE VANDENBROUCK,‡
AMADEU M.V.M. SOARES,† and WIM DE COEN‡
†CESAM and Department of Biology, University of Aveiro, Aveiro, Portugal
‡Department of Biology, University of Antwerp, Antwerp, Belgium

OPEN ACCESS Freely available online



2 *Enchytraeus albidus* Microarray: Enrichment, Design, Annotation and Database (EnchyBASE)

Sara C. Novais^{1*}, Joel Arrais², Pedro Lopes², Tine Vandenbrouck³, Wim De Coen³, Dick Roelofs⁴,
Amadeu M. V. M. Soares¹, Mónica J. B. Amorim¹

April 2012 | Volume 7 | Issue 4 | e34266



- Platform: Agilent
- μ array: 8 x 15K
- No seq: 2100

3

Castro-Ferreira et al. BMC Genomics 2014, 15:302
http://www.biomedcentral.com/1471-2164/15/302



RESEARCH ARTICLE

Open Access

Transcriptome assembly and microarray construction for *Enchytraeus crypticus*, a model oligochaete to assess stress response mechanisms derived from soil conditions

Marta P. Castro-Ferreira^{1,2}, Tjalf E. de Boer¹, John K. Colbourne^{3,4}, Riet Vooijs¹, Cornelis AM van Gestel¹,
Nico M van Straalen¹, Amadeu MVM Soares², Mónica JB Amorim² and Dick Roelofs^{1*}



- Platform: Agilent
- μ array: 4 x 44K
- No seq: 43 749



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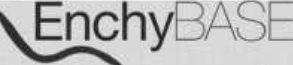


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Tools: development of Enchybase

<http://bioinformatics.ua.pt/enchybase/>

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Background | Search | **BLAST** | Microarray Data | Publications

BASIC SEARCH

Default BLAST parameter settings

Enter query sequences here in Fasta format

Or upload sequence fasta file for querying: No file chosen

Program Libraries

All Enchytraeus albidus
Normalized Enchytraeus albidus
Metals Enchytraeus albidus
Pesticides Enchytraeus albidus

ADVANCED SEARCH

Set your favourite parameters below

Expect threshold

Word size

[bioinformatics.ua.pt/enchybase/#blast](#)

Information

- EnchyBASE is constructed using PartGene software.
- EnchyBASE contains ESTs from the enchytraeid species.
 - *Enchytraeus albidus*
 - Normalized library - 348 ESTs
 - Library enriched for metals (SSH) - 875 ESTs
 - Library enriched for pesticides (SSH) - 877 ESTs
 - *Enchytraeus crypticus*
 - Available soon

Links



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Available genomic tools in soil ecotox

Enchytraeus albidus: 2100 EST

Agilent 8 x 15K μ array

Novais et al. 2012 (PlosOne, 7:4)



Eisenia fetida: 3144 EST

Pirooznia et al. 2007 (BMC Bioinformatics, 8)

Custom made μ array: 4K



Folsomia candida: 8686 EST

Timmermans et al. 2007 (BMC Genomics, 8:341)

Agilent 2 x 11K μ array



Lumbricus rubellus: 17225 EST

Owen et al. 2008 (BMC Genomics, 9:266)

Custom made μ array: 8K



Enchytraeus crypticus: >114000 seq

Castro-Ferreira et al. 2012 (BMC Genomics, 15:302)

Agilent 4 x 44K μ array



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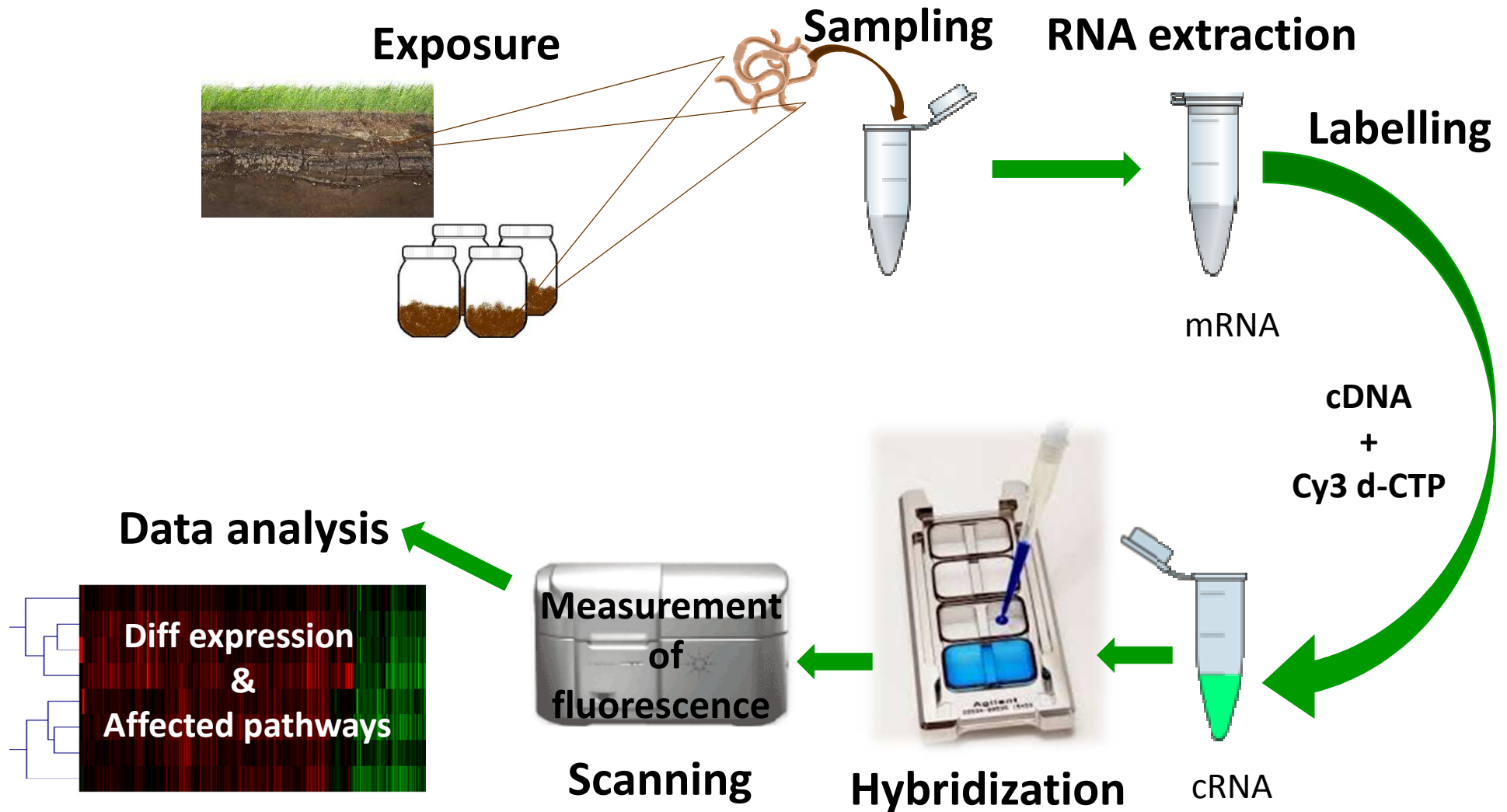
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Methodology



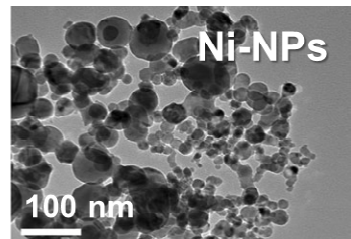
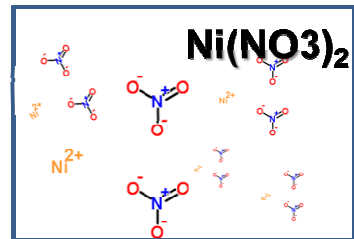
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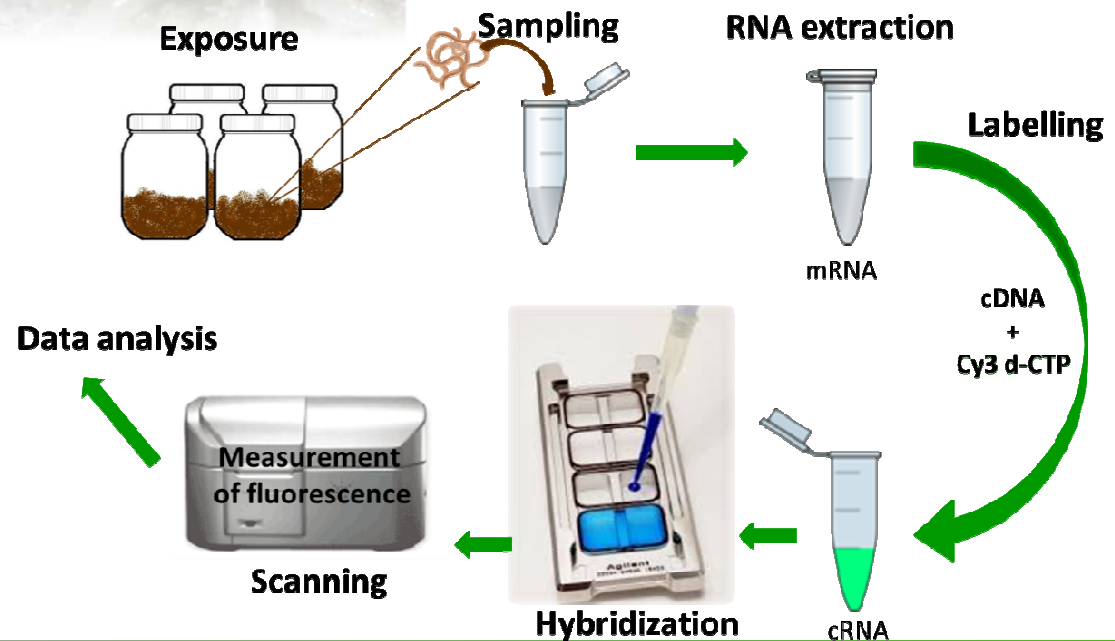
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Case study

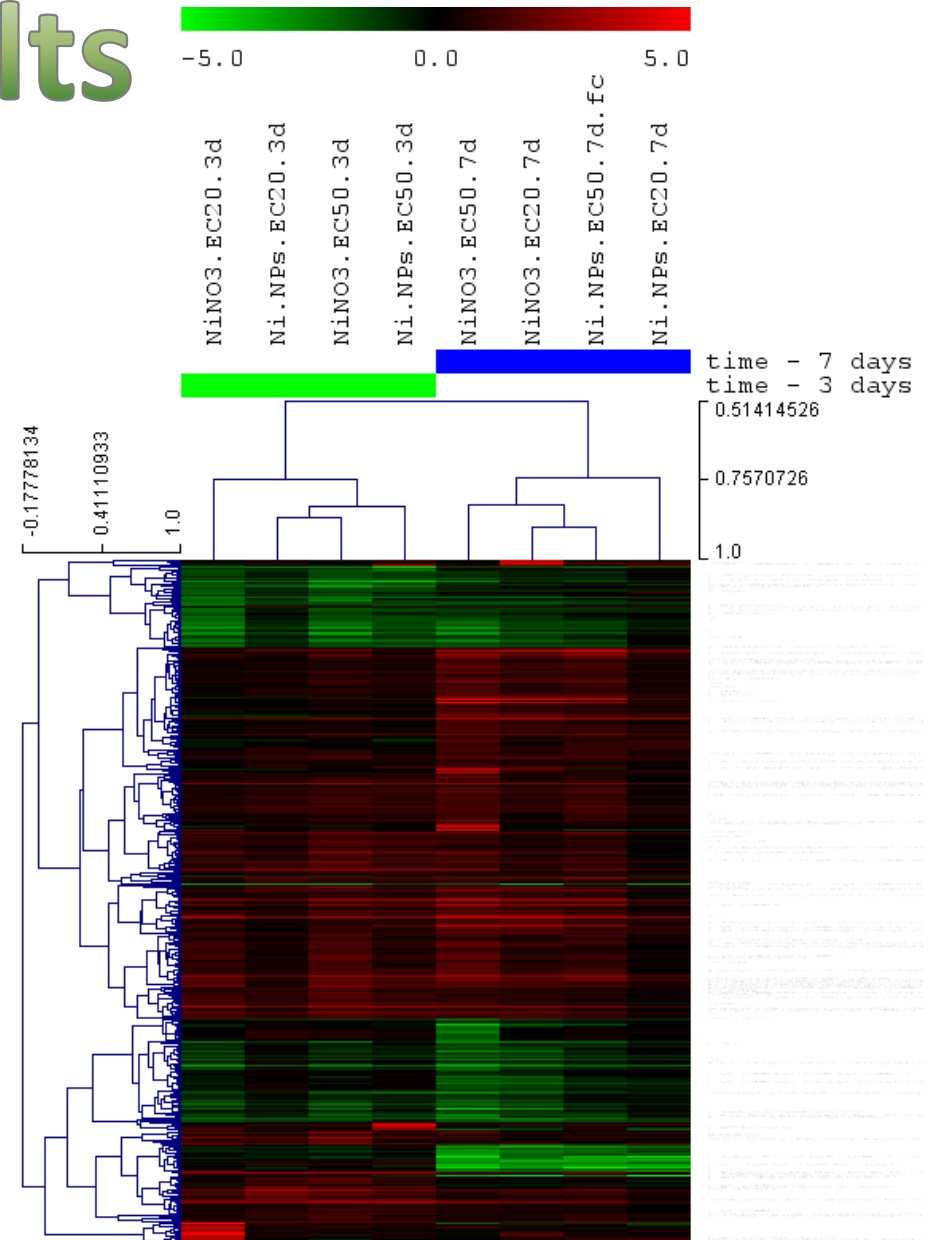
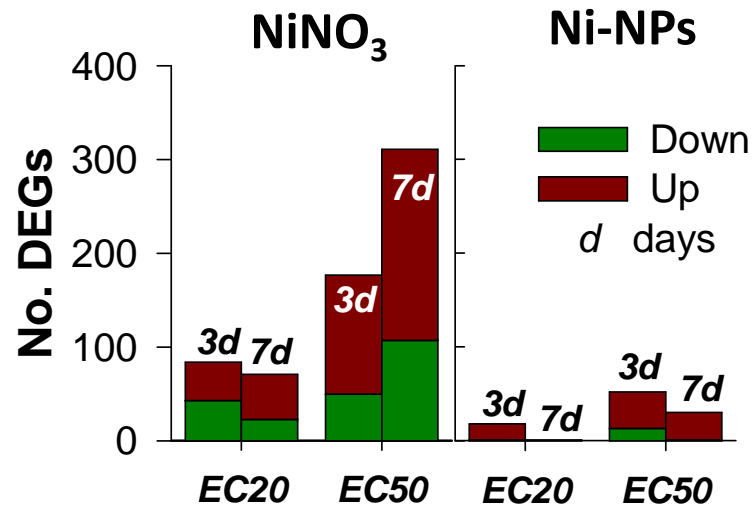


Effect Concentrations (mg/kg)

	EC ₂₀	EC ₅₀
NiNO ₃	40	60
Ni-NPs	980	1760



Case study: results



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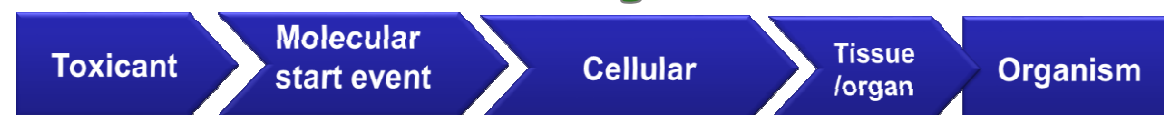
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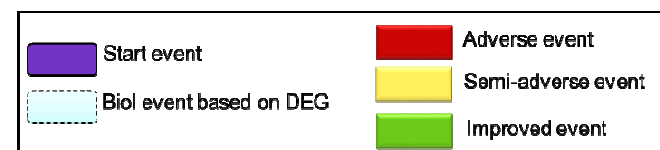
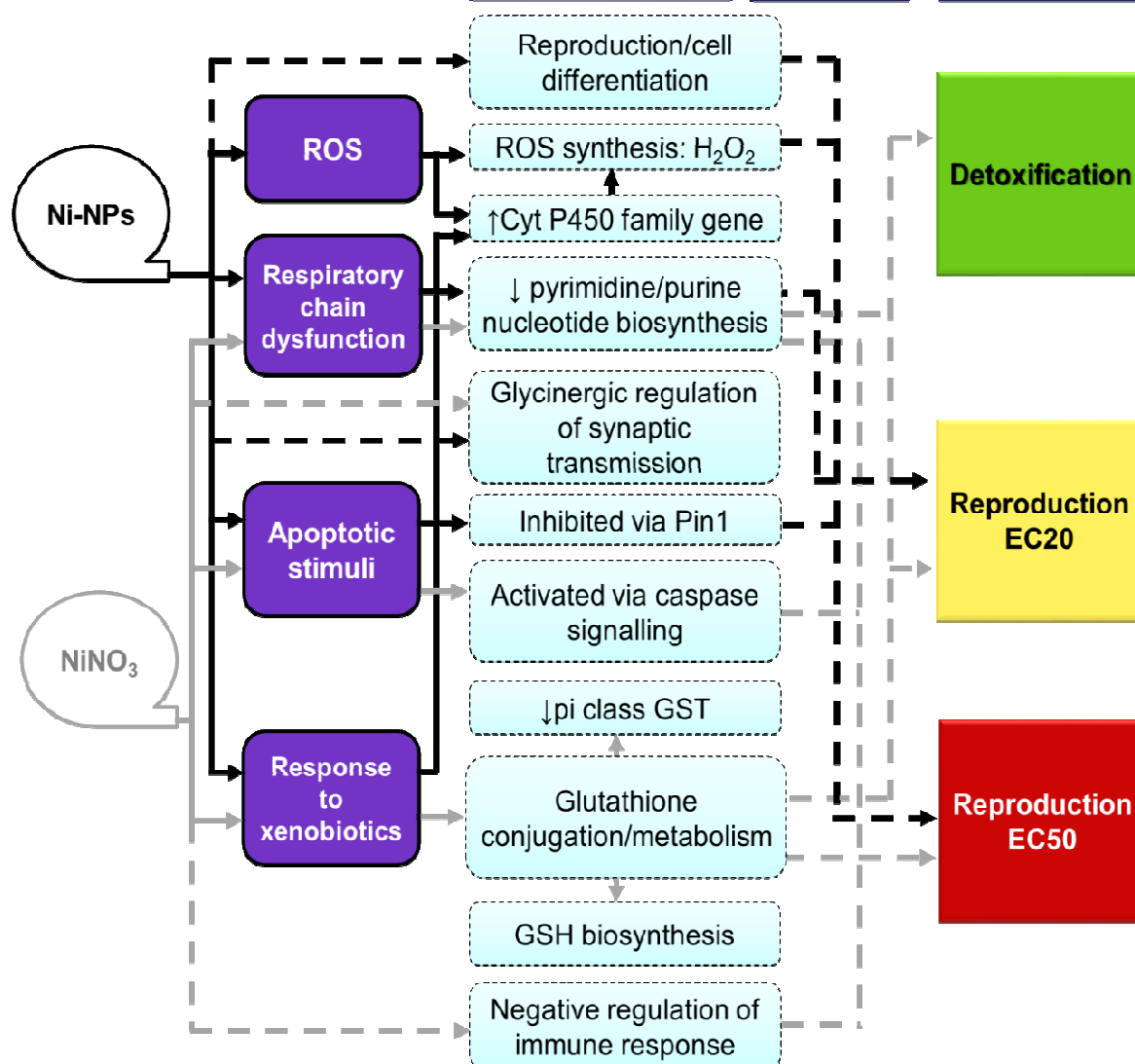
Case study: results integration



AOP

Identification of materials specific mechanisms of action, e.g.:

- **Ni-NPs specific:**
ROS synthesis (with involvement of a Cyt P450 family gene);
Reproduction and cell differentiation
- **Ni-salt specific:**
Glutathione metabolism and conjugation;
Negative regulation of immune response



Output

- **High-throughput tool** that allows the identification of hypothesis, i.e., targeted sequential test design.
- **Identification of materials MoA**
 - anchored with effects at organism level
- **Differentiation between Ni-forms**
(not possible based on standard ecotox tests)



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Thank You



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