

# Bioassays and tools for soil quality assessment

Contribuições do CESAM para o Uso Inteligente das Funções do Solo

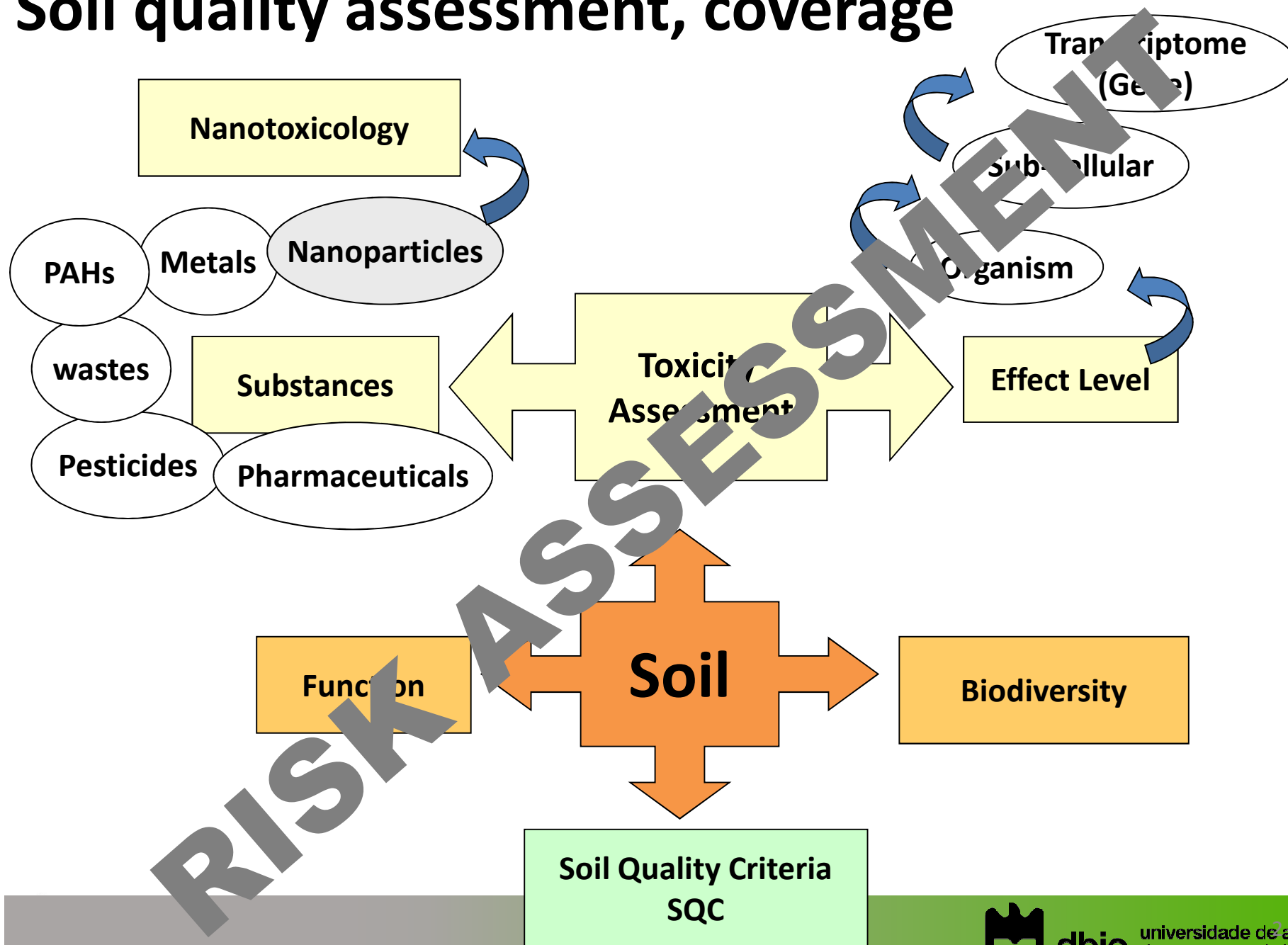
10 maio 2016, Edifício III, Universidade de Aveiro

\*Obrigatório



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# Soil quality assessment, coverage



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universidade de aveiro  
departamento de biologia

# Soil quality assessment

1. **Standardised test methods** for the assessment of contaminated soils – a compilation
2. **Advanced methods** (examples)
3. **Sub-organism tools**
4. **Overview and strategies**

# 1. Standardised test methods: PLANTS

|                   |  |
|-------------------|--|
| <b>Name:</b>      | Terrestrial plant test   |
| <b>Guideline:</b> | OECD 208 A,B (2006)  |
| <b>Species:</b>   | <i>Brassica rapa</i> , <i>Avena sativa</i> or 27 other mono- and dicotyledone crop species |
| <b>Substrate:</b> | Field soils (e.g. LUFA), artificial soil   |
| <b>Duration:</b>  | Approximately four weeks   |
| <b>Parameter:</b> | Seedling emergence and growth, biomass   |
| <b>Design:</b>    | ECx (about 24 vessels), limit test   |



# 1. Standardised test methods: PLANTS

## Effects of zinc-contaminated soil on *B. rapa*





# 1. Standardised test methods: PLANTS

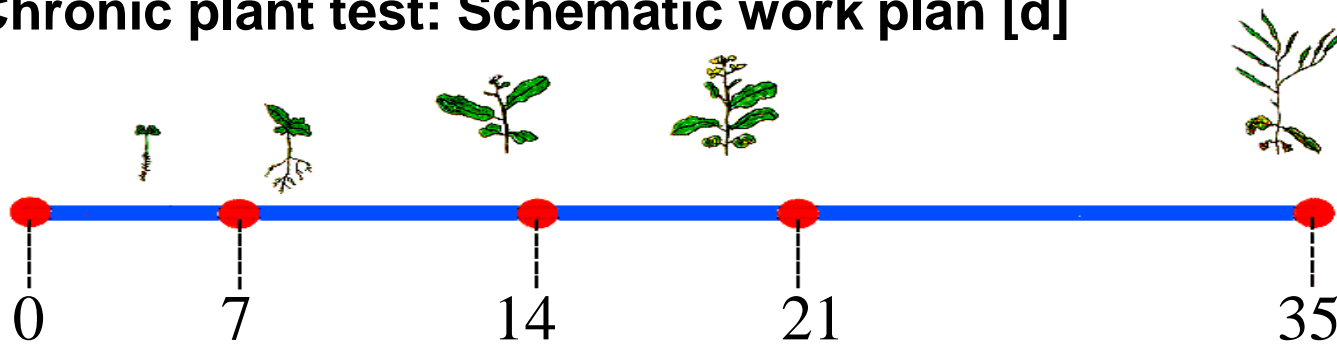
Growth of  
*A. sativa*  
(control)



# 1. Standardised test methods: PLANTS

|                   |   |
|-------------------|---|
| <b>Name:</b>      | Chronic toxicity in higher plants                   |
| <b>Guideline:</b> | ISO 22030 (2005);<br>comparable to ASTM (1991)      |
| <b>Species:</b>   | <i>B. rapa</i> (rapid cycling), <i>Avena sativa</i> |
| <b>Substrate:</b> | Field soils, artificial soil                        |
| <b>Duration:</b>  | 5 – 8 weeks (depending on species)                  |
| <b>Parameter:</b> | Biomass, number of flowers and/or seed pods         |
| <b>Design:</b>    | NOEC, EC <sub>x</sub> (24 – 30 vessels)             |

## Chronic plant test: Schematic work plan [d]



sowing  
(10 seeds  
per vessel)

thinning  
(8 seedlings)

harvesting  
(4 plants)

pollination

harvesting  
(4 plants)

# 1. Standardised test methods: PLANTS

## Development of *B. rapa*: Day 5 and Day 35





# 1. Standardised test methods: “SAPROS”

|                    |   |
|--------------------|---|
| <b>Name:</b>       | <b>Earthworm acute test</b>                               |
| <b>Guideline:</b>  | ISO 11268-1 (1993), OECD 207 (1984)                       |
| <b>Species:</b>    | <i>Eisenia fetida</i> , <i>E. andrei</i>                  |
| <b>Substrate:</b>  | Artificial Soil or field soils, e.g. LUFA                 |
| <b>Duration:</b>   | 14 days   |
| <b>Parameter:</b>  | Mortality, biomass  |
| <b>Design:</b>     | NOEC (24 vessels), limit test                             |
| <b>Experience:</b> | Various differently contaminated soils (plus a ring-test) |

## ***E. fetida* in artificial and field soil**



# 1. Standardised test methods : “SAPROS”

|                    |   |
|--------------------|---|
| <b>Name:</b>       | <b>Earthworm reproduction test</b>                        |
| <b>Guideline:</b>  | ISO 11268-2 (1998), OECD 222 (draft)                      |
| <b>Species:</b>    | <i>Eisenia fetida</i> , <i>E. andrei</i>                  |
| <b>Substrate:</b>  | Artificial Soil or field soils, e.g. LUFA                 |
| <b>Duration:</b>   | 56 days   |
| <b>Parameter:</b>  | Biomass, number of juveniles                              |
| <b>Design:</b>     | NOEC (24 vessels), limit test                             |
| <b>Experience:</b> | Various differently contaminated soils (plus a ring-test) |

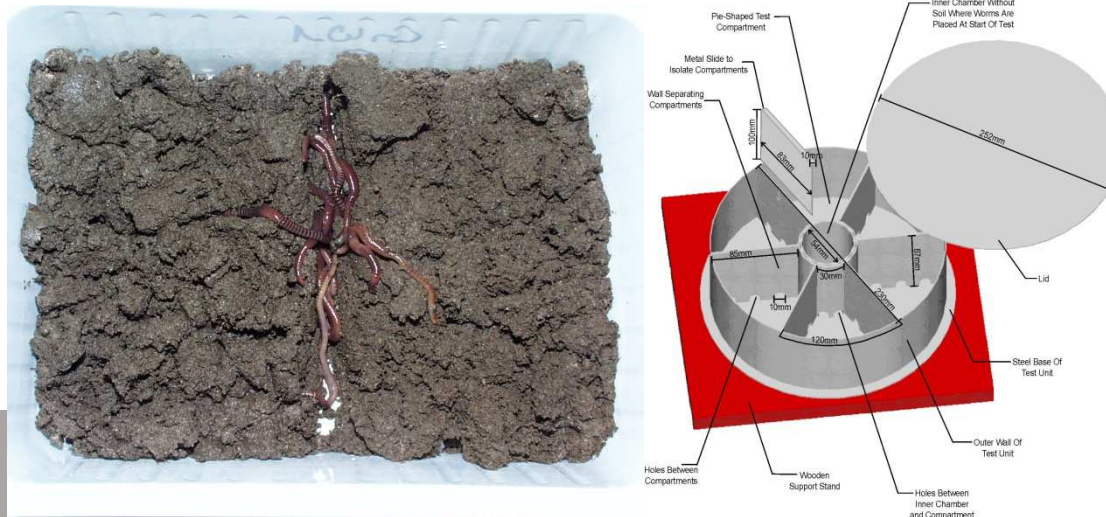
**Counting of juveniles  
in a water-bath**



# 1. Standardised test methods : “SAPROS”

|                    |  |
|--------------------|--|
| <b>Name:</b>       | Earthworm avoidance test   |
| <b>Guideline:</b>  | ISO 17512-1 (2008)   |
| <b>Species:</b>    | <i>Eisenia fetida</i> , <i>E. andrei</i>                                     |
| <b>Substrate:</b>  | Artificial Soil or field soils, e.g. LUFA                                    |
| <b>Duration:</b>   | 2 days   |
| <b>Parameter:</b>  | Behaviour  |
| <b>Design:</b>     | NOEC, ECx (number of vessels depending on the design (dual or six sections)) |
| <b>Experience:</b> | Tests with various different soils and contaminations                        |

## Avoidance test: Dual and six section design



# 1. Standardised test methods: “SAPROS”

|                    |   |
|--------------------|---|
| <b>Name:</b>       | <b>Earthworm field test</b>   |
| <b>Guideline:</b>  | ISO 11268-3 (1999), BBA (1994)  |
| <b>Species:</b>    | Natural community; in particular <i>Aporrectodea caliginosa</i> , <i>Lumbricus terrestris</i> (temperate regions) |
| <b>Substrate:</b>  | Field sites   |
| <b>Duration:</b>   | Up to 12 months   |
| <b>Parameter:</b>  | Abundance, biomass, species composition   |
| <b>Design:</b>     | Limit test (12 plots)   |
| <b>Experience:</b> | Very few in Europe  |

**Epigeic, endogeic and anecic earthworms**





# 1. Standardised test methods: “SAPROS”

|                    |  |
|--------------------|--|
| <b>Name:</b>       | Enchytraeid reproduction test                                    |
| <b>Guideline:</b>  | ISO 16387 (2002), OECD 220 (2004)                                |
| <b>Species:</b>    | <i>E. albidus</i> , <i>E. crypticus</i> , <i>Enchytraeus</i> sp. |
| <b>Substrate:</b>  | Artificial Soil or field soils, e.g. LUFA                        |
| <b>Duration:</b>   | Up to six weeks  |
| <b>Parameter:</b>  | Mortality, number of juveniles                                   |
| <b>Design:</b>     | NOEC, ECx (28 – 30 vessels)                                      |
| <b>Experience:</b> | Wide range of differently contaminated soils (two ring-tests)    |





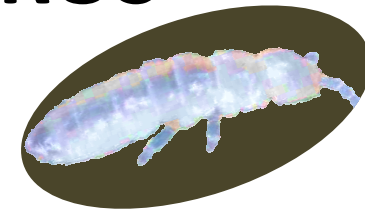
# 1. Standardised test methods: “SAPROS”

## Biology of Enchytraeids:

- Small (1 – 40 mm length)  
mainly whitish worms
- Important for OM breakdown
- About 300 species in Europe
- World-wide distribution
- In soil: 20.000 - 60.000  
ind/m<sup>2</sup> (annual mean)

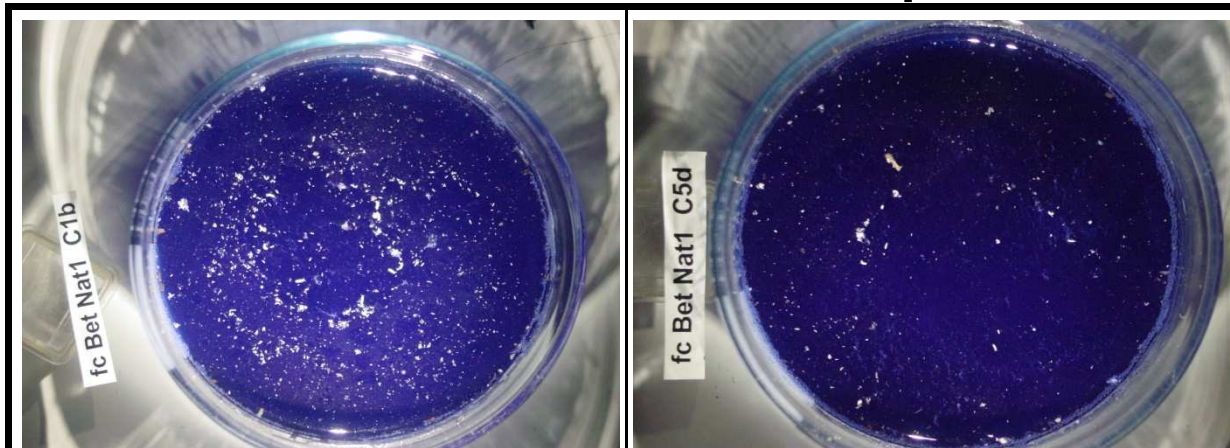


# 1. Standardised test methods: “SAPROS”



|                    |   |
|--------------------|---|
| <b>Name:</b>       | Collembolan reproduction test                                   |
| <b>Guideline:</b>  | ISO 11267 (1999)  |
| <b>Species:</b>    | <i>Folsomia candida</i>   |
| <b>Substrate:</b>  | Artificial soil or field soils, e.g. LUFA                       |
| <b>Duration:</b>   | 28 days   |
| <b>Parameter:</b>  | Mortality, number of juveniles                                  |
| <b>Design:</b>     | NOEC, ECx (30 vessels)  |
| <b>Experience:</b> | Wide range of differently contaminated soils (plus a ring-test) |

**Reference substance: Effects on reproduction**



**Control**

**Betanal (a.i. Phenmedipharm):**

**10 mg/kg**

applEE



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# 1. Standardised test methods: “SAPROS”

|                    |   |
|--------------------|---|
| <b>Name:</b>       | Chronic toxicity to dung larvae   |
| <b>Guideline:</b>  | OECD 122 (2010)   |
| <b>Species:</b>    | <i>Aphodius constans</i>  |
| <b>Substrate:</b>  | Artificial soil or field soil plus dung                                 |
| <b>Duration:</b>   | Six weeks   |
| <b>Parameter:</b>  | Mortality, biomass  |
| <b>Design:</b>     | NOEC, ECx (24 – 30 vessels)   |
| <b>Experience:</b> | Developed for veterinary drugs; relevance for site assessment not clear |

## Dung beetle in their natural habitat



# 1. Standardised test methods: PREDATOR

|                   |                                  |
|-------------------|----------------------------------|
| <b>Name:</b>      | Predatory mite reproduction test |
| <b>Guideline:</b> | OECD 226 (2008)                  |
| <b>Species:</b>   | <i>Hypoaspis aculeifer</i>       |
| <b>Substrate:</b> | Field soil or artificial soil    |
| <b>Duration:</b>  | Three weeks                      |
| <b>Parameter:</b> | Mortality, reproduction          |
| <b>Design:</b>    | NOEC (28 vessels)                |

**H. aculeifer: Life stages**



**Feeding behaviour**



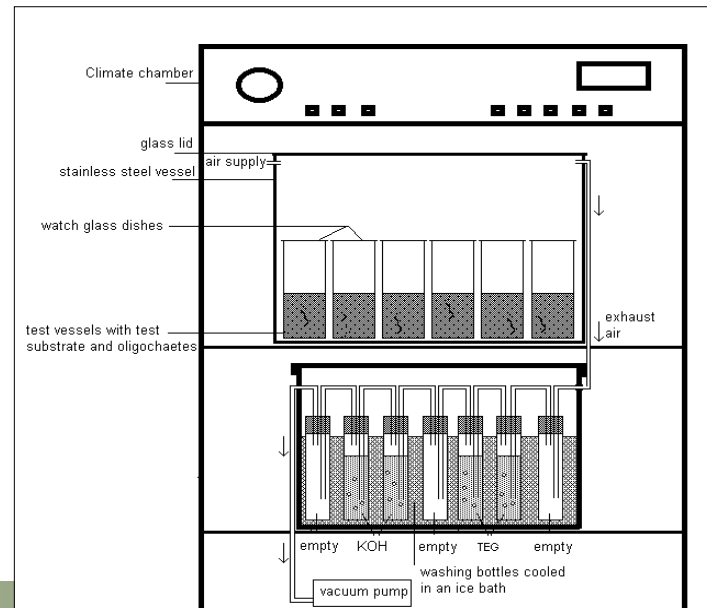


# 1. Standardised test methods: Bioaccumulation

|                   |  |
|-------------------|--|
| <b>Name:</b>      | Oligochaete bioaccumulation test   |
| <b>Guideline:</b> | OECD 317 (2010)  |
| <b>Species:</b>   | <i>Eisenia fetida</i> , <i>E. andrei</i> , <i>Lumbricus rubellus</i> , <i>E. albidus</i> or <i>E. luxuriosus</i> |
| <b>Substrate:</b> | Artificial Soil or field soils, e.g. LUFA  |
| <b>Duration:</b>  | At least 28 - 42 days  |
| <b>Parameter:</b> | Bioaccumulation factor: BAF or BSAF (lipid-normalised)   |
| <b>Design:</b>    | Uptake and elimination phase   |

## Test Set-up

### Test chamber

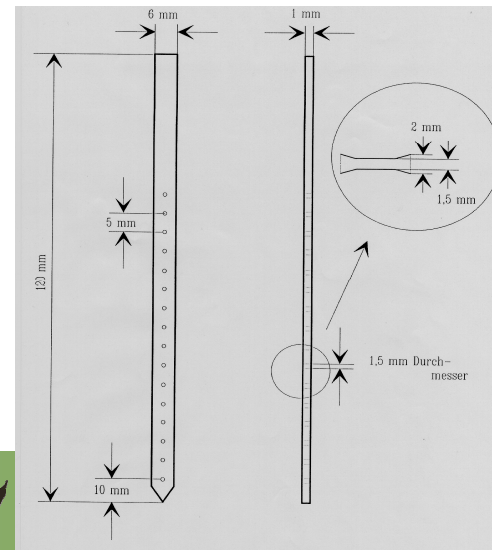




# 1. Test methods: Functional

|                    |   |
|--------------------|---|
| <b>Name:</b>       | <b>Bait-lamina test</b>   |
| <b>Guideline:</b>  | Proposal from literature (Törne 1990)                                 |
| <b>Principle:</b>  | Feeding activity (no distinction between microbes and invertebrates)  |
| <b>Substrate:</b>  | Field sites / soils (world-wide)                                      |
| <b>Duration:</b>   | Usually between 4 days and 4 weeks                                    |
| <b>Parameter:</b>  | Fed / non-fed holes visually assessed                                 |
| <b>Design:</b>     | Treatment versus control  |
| <b>Experience:</b> | Limited experience (UK, Germany); can be included in laboratory tests |

## Fed / non fed holes (terra protecta®)



# 1. Test methods: Functional

**Block of bait-lamina  
in the field**



**Bait-lamina in an  
earthworm repro test**



# 1. Test methods: Functional

|                   |  |
|-------------------|--|
| <b>Name:</b>      | Breakdown of organic matter in Litter bag test |
| <b>Guideline:</b> | OECD 56 (2006)                                 |
| <b>Principle:</b> | Organic matter breakdown                       |
| <b>Substrate:</b> | Field sites / soils (world-wide)               |
| <b>Duration:</b>  | Usually between 6 and 12 months                |
| <b>Parameter:</b> | Mass loss of (e.g.) wheat straw                |
| <b>Design:</b>    | Treatment versus control                       |

**Litter bags and mini-container in the litter layer**



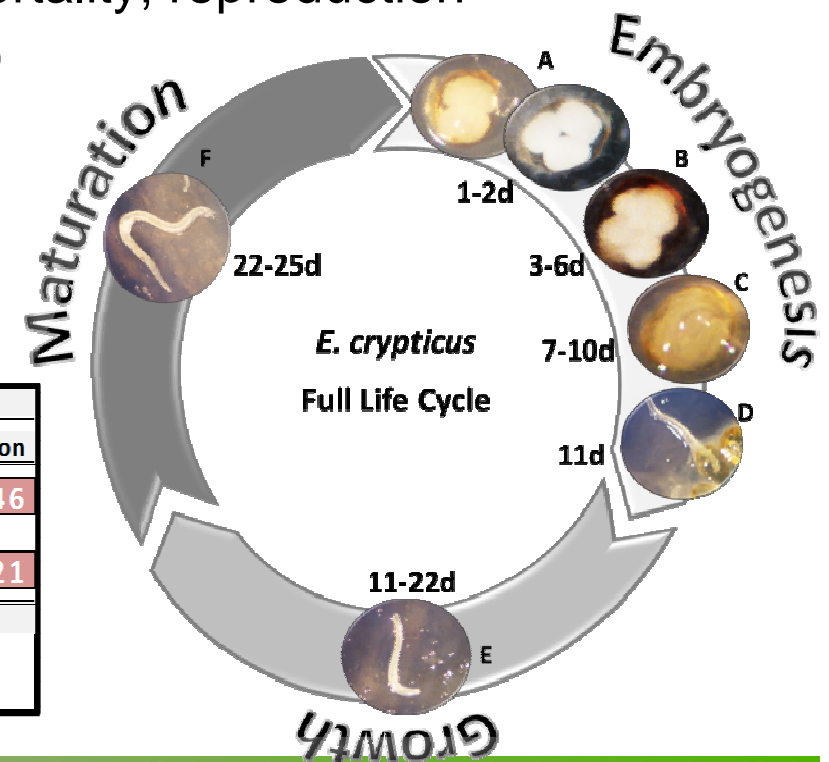
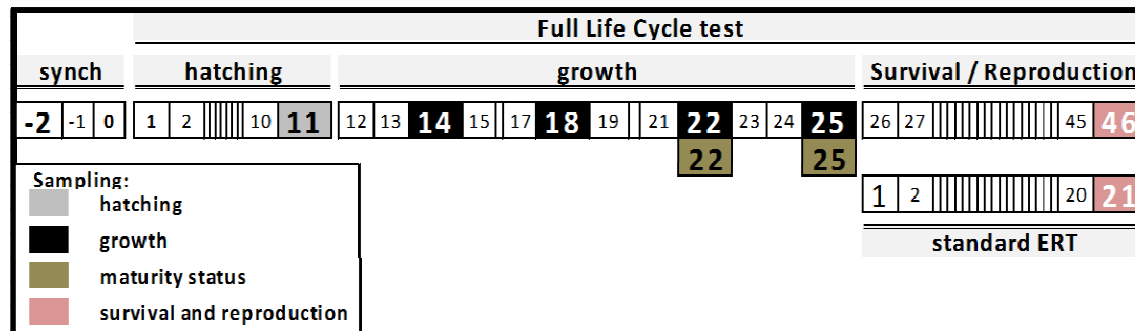
**Wheat-filled buried bag**





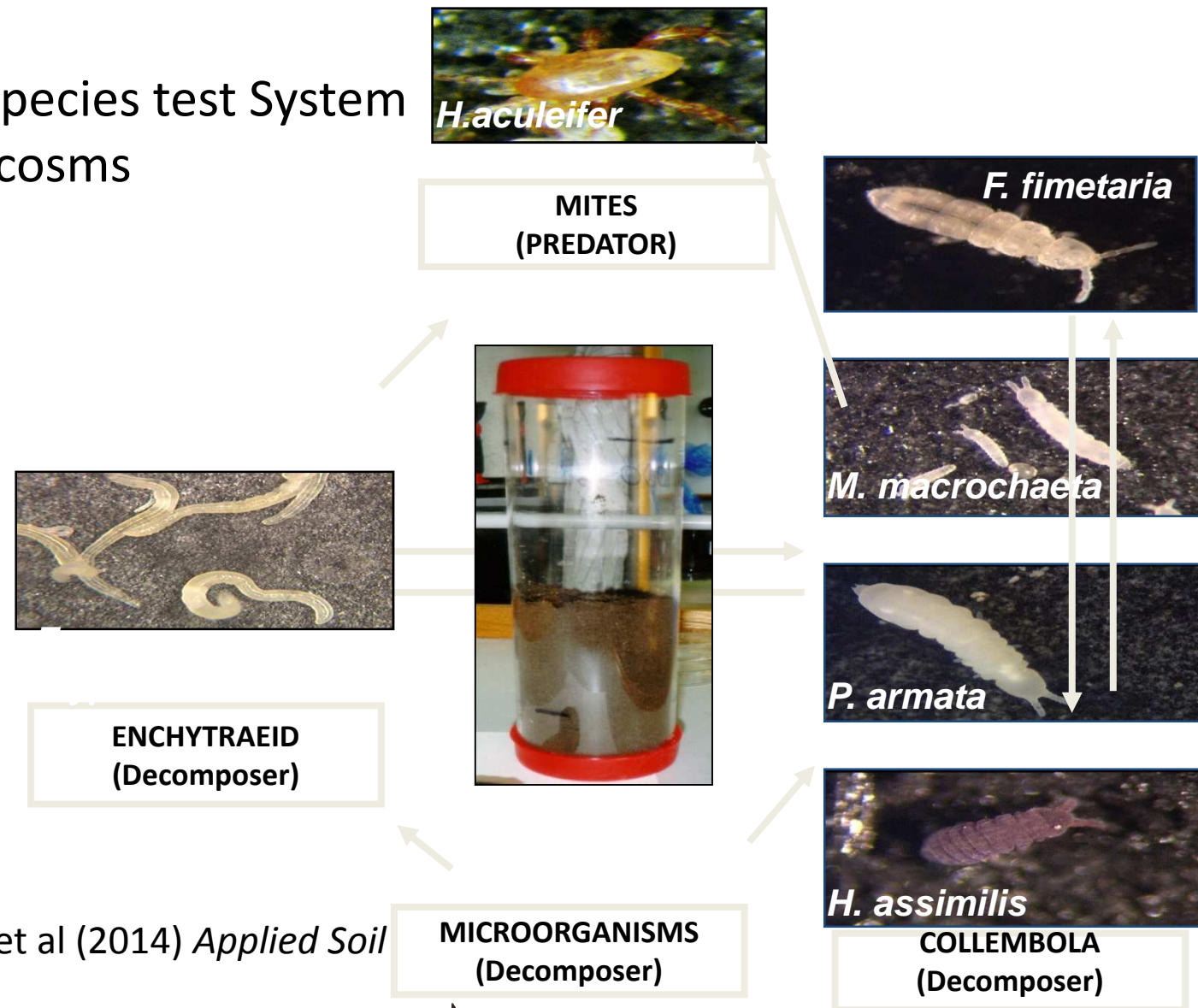
## 2. Advanced methods: Full life cycle

|                   |   |
|-------------------|---|
| <b>Name:</b>      | <b>Enchytraeid full life cycle test</b>                       |
| <b>Status:</b>    | published: Bicho et al (2015) Ecotoxicology. 24, 5, 1053-1063 |
| <b>Species:</b>   | <i>E. crypticus</i>   |
| <b>Substrate:</b> | Artificial Soil or field soils, e.g. LUFA                     |
| <b>Duration:</b>  | Up to 46 days   |
| <b>Parameter:</b> | hatching, growth, maturity, mortality, reproduction           |
| <b>Design:</b>    | NOEC, ECx (28 – 30 vessels)                                   |



## 2. Advanced methods: Multispecies/ Mesocosms

**SMS:** Soil Multispecies test System  
- Built soil mesocosms



Menezes-Oliveira V.B. et al (2014) *Applied Soil Ecology*. 75, 24–32



## 2. Advanced methods: Multispecies/ Mesocosms

**TME:** Terrestrial Model Ecosystems

- Field collected soil core



Morgan and Knacker (1994). Ecotoxicology. 3(4):213-233

### 3. Sub-organism tools: biomarkers

- **General definition:**

A biological response to a chemical or chemicals that gives a measure of exposure and, sometimes, also of toxic effect (Peakall 1994)

- **Often used in a more specific way:**

Tests systems focussing on sub-organismic level:  
Biochemical, molecular, physiological and genetic

- **Examples:**

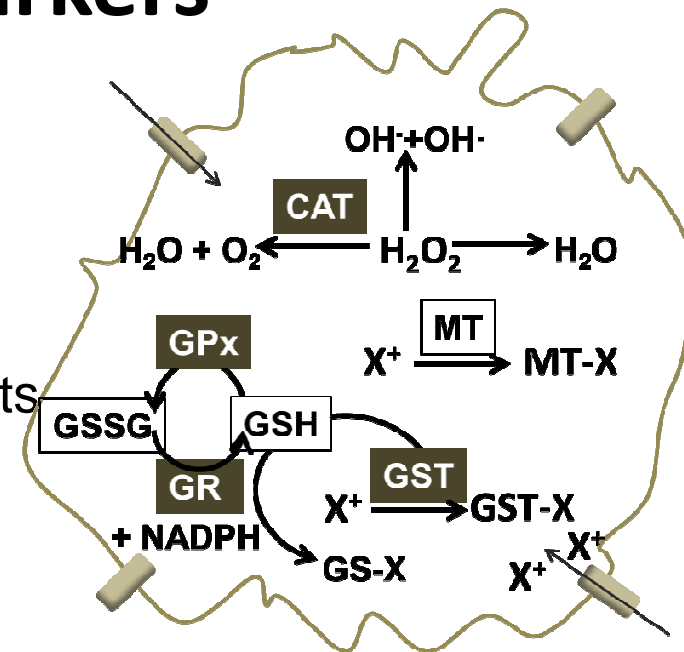
- **oxidative stress biomarkers** (enzymatic)
- **transcriptomics** (and other -omics)
- **epigenetic markers** (transgenerational)

### 3. Sub-organism tools: biomarkers

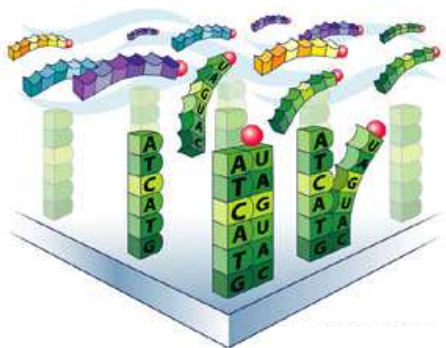
- Oxidative stress markers
  - Optimized for several soil organisms
  - Enzymatic and non-enzymatic endpoints

- Transcriptomics

- differential gene expression
- assess thousands of genes simultaneously (high-throughput)
- national microarrays facility at UAVR
- developed at UAVR for e.g. *Enchytraeus crypticus*



Ribeiro et al, 2015



appliance



Agilent Technologies

eArray



# 4. Soil quality assessment: overview

|                                      | ENDPOINT                  | n.º species      | OECD / ISO | throughput/ detail | exposure time | sensitivity | skills/ equipment | Effect      |             |            |
|--------------------------------------|---------------------------|------------------|------------|--------------------|---------------|-------------|-------------------|-------------|-------------|------------|
|                                      |                           |                  |            |                    |               |             |                   | Performance | Mechanistic | Functional |
| sub-Organism (sub-O):                |                           | NON-PHENOTYPICAL |            |                    |               |             |                   |             |             |            |
|                                      | Molecular:                |                  |            |                    |               |             |                   |             |             |            |
| 1                                    | Genomic                   | 2                | 1          | 5                  | 5             | 5           | 1                 |             | M           |            |
| 2                                    | Transcriptomic            | >5               | 1          | 5                  | 5             | 5           | 2                 |             | M           |            |
| 3                                    | Proteomic                 | 1                | 1          | 5                  | 4             | 5           | 1                 |             | M           |            |
| 4                                    | Metabolomic               | 1                | 1          | 5                  | 5             | 5           | 1                 |             | M           |            |
| 5                                    | Epigenetic                | 1                | 1          | 3                  | 3             | 5           | 2                 |             | M           | F          |
| 6                                    | Biomarkers*               | >5               | 1          | 4                  | 4             | 4           | 2                 | P           | M           | F          |
|                                      | Cellular/Tissue:          |                  |            |                    |               |             |                   |             |             |            |
| 7                                    | Cell uptake mech.#        | >5               | 1          | 4                  | 4             | 4           | 2                 |             | M           | F          |
| 8                                    | Cell viability            | >5               | 1          | 4                  | 5             | 4           | 2                 | P           | M           | F          |
| 9                                    | Histology                 | >5               | 1          | 3                  | 3             | 3           | 3                 |             | M           | F          |
| 10                                   | Embryo development        | 1                | 1          | 3                  | 5             | 4           | 3                 | P           | M           |            |
| Organism (O):                        |                           | PHENOTYPICAL     |            |                    |               |             |                   |             |             |            |
| 11                                   | Bioaccumulation/ kinetics | >5               | 4          | 4                  | 2             | 4           | 4                 | P           | M           |            |
| 12                                   | Avoidance                 | 3                | 5          | 2                  | 5             | 2           | 4                 | P           |             |            |
| 13                                   | Survival                  | >5               | 5          | 1                  | 4             | 1           | 4                 | P           |             |            |
| 14                                   | Growth                    | >5               | 5          | 3                  | 3             | 2           | 4                 | P           |             |            |
| 15                                   | Reproduction              | >5               | 5          | 3                  | 2             | 3           | 4                 | P           |             |            |
| 16                                   | Full Life Cycle           | ≤2               | 4          | 4                  | 1             | 4           | 4                 | P           | M           |            |
|                                      | Community:                |                  |            |                    |               |             |                   |             |             |            |
| 17                                   | Trophic Transfer          | 1                | 1          | 2                  | 1             | 3           | 4                 | P           |             | F          |
| 18                                   | Multigenerational         | 1                | 1          | 4                  | 1             | 4           | 4                 | P           | M           |            |
| 19                                   | Multispecies/mesocosms    | 1                | 1          | 4                  | 1             | 4           | 4                 | P           |             | F          |
| Sub-Organism & Organism (Sub-O & O): |                           |                  |            |                    |               |             |                   |             |             |            |
| 20                                   | System Toxicology         | 5                |            | 5                  | 1-5           | 5           | 1-5               | P           | M           | F          |

**P:** Performance;

**M:** Mechanistic;

**F:** Functional

Colour aims to illustrate negative (red) to positive (green) classification.

Environmental Pollution xxx (2015) 1–6



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Commentary

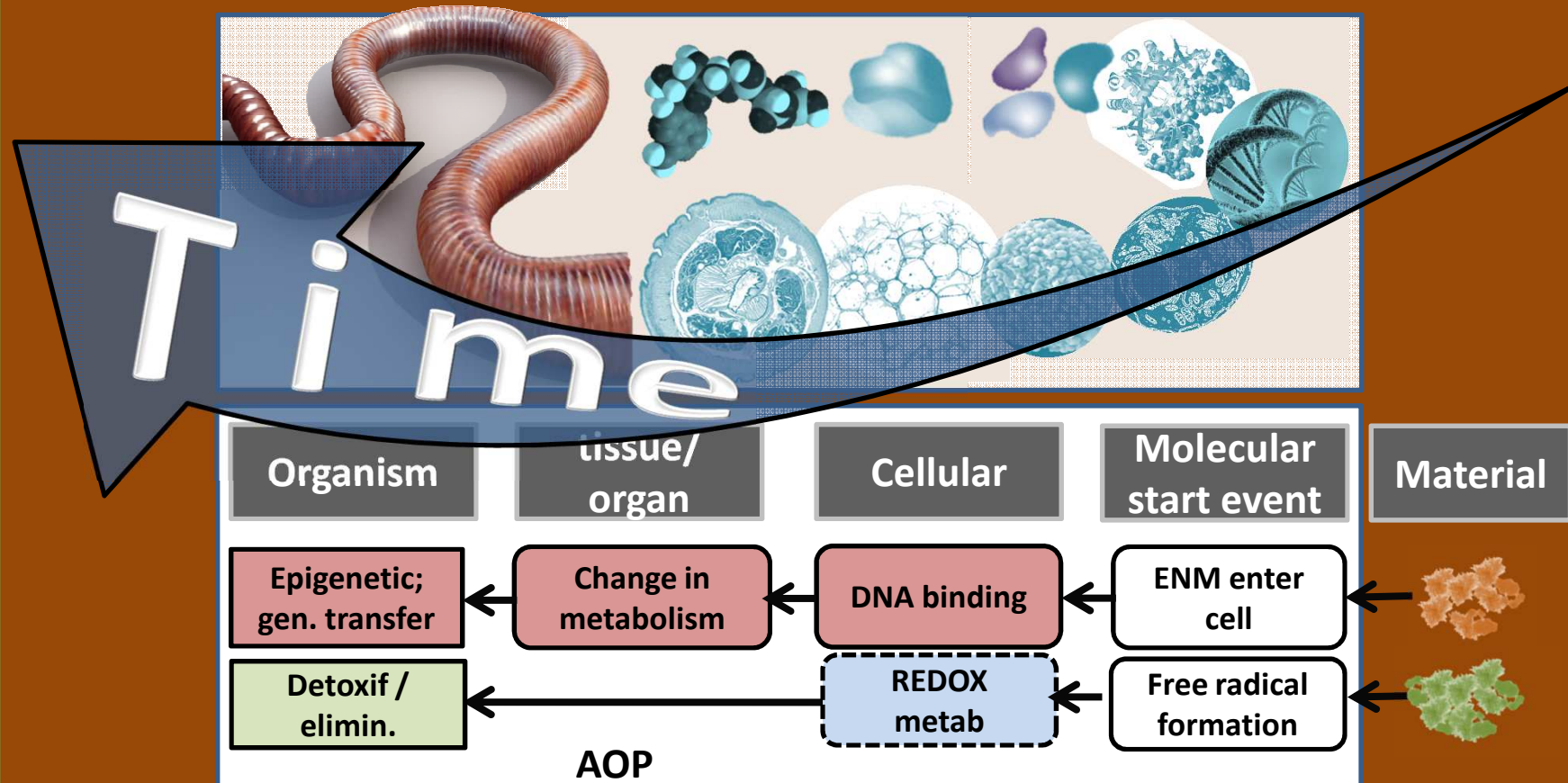
Effect assessment of engineered nanoparticles in solid media – Current insight and the way forward

Mónica J.B. Amorim <sup>a,\*</sup>, Carlos P. Roca <sup>c</sup>, Janeck J. Scott-Fordsmand <sup>b</sup>

aveiro  
biologia

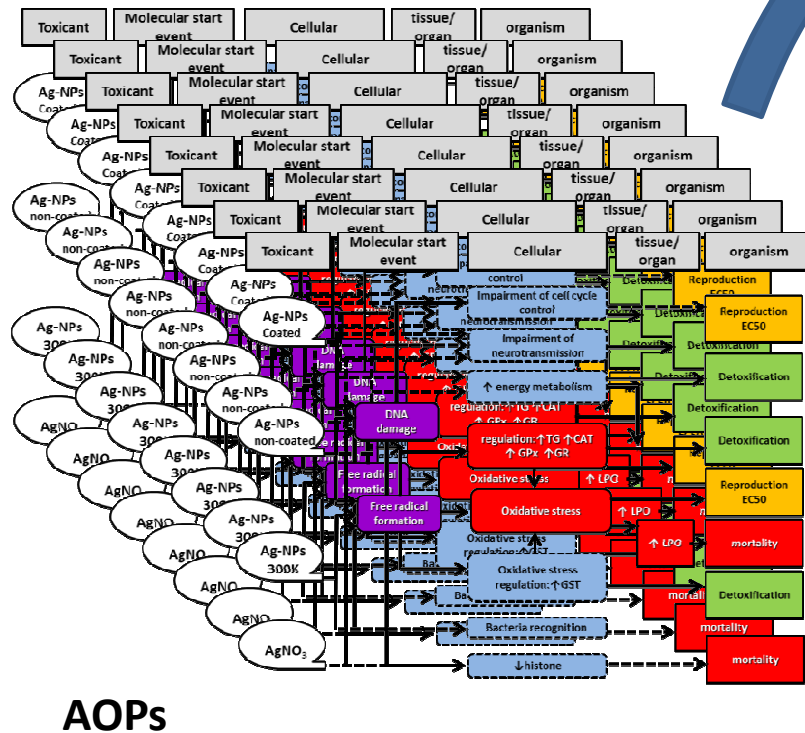


## 4. Soil quality assessment: strategy



**Adverse Outcome Pathways**

# Finally..



- inside to potential effects
- knowledge for grouping / ranking
- enable safer-by-design
- support a KBRA and ITS




**Effects**  
**LONG TERM ← SHORT TERM**

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The screenshot shows the CESAM website header with the logo and navigation menu. Below the header, a sidebar lists various categories like Thematic Lines, Research Groups, and Members. The main content area features a profile for Mónica Amorim, including her photo, contact information, and research details.

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Category: Researcher  
Department: Biology  
Research group: Stress Biology  
ISI Web of Knowledge<sup>SM</sup> search factor: AU=(Amorim M B or Amorim M or Amorim M D)  
AND AD=(Aveiro or Coimbra); Research ID = G-8590-2011; ORCID <http://orcid.org/0000-0001-8137-3295>  
Direct URL for this page: <http://www.cesam.ua.pt/monicaamorim>