Using the DEBkiss model to integratively assess effects of tributyltin on the freshwater gastropod *Lymnaea stagnalis*.

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• The endocrine system regulates the metabolism and function of the body

• An endocrine disrupter (EDC) is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations (WHO/IPCS 2002)
Which chemicals are EDCs?
• certain pesticides/biocides (DDT)
• industrial chemicals (PCBs, bisphenol A)
• plant hormones (phytoestrogens)

Why EDCs are of concern?
• alterations of hormonal balance (energy)
• reproductive abnormalities (early puberty, affect fertility and fecundity, male/female ratio)
• developmental impairment
• diseases
• behavioural changes
• teratogenic effects...
• **Directive on PPP (EC 1107/2009):** marketing and use of chemical products shall be approved only if they do not have endocrine disrupting properties that may cause adverse effects on *populations* of non-target organisms under realistic conditions.

• Constrains for practical application of the directive:
  - scientific criteria for identifying EDs properties are not agreed yet
  - terms “adverse effects” and “realistic conditions” are vague
  - the population effects need to be assess
  - standard guidelines are needed
OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals (revised in 2012)

- Standardisation of toxicity tests with molluscs is on the way
Effects of TBT in molluscs:
• imposex – superimposition of male sex organs on a female, registered in more than 200 gastropod species (Europe, US, Japan, South America)
• intersex
• sterilisation of females
• shell abnormal thickening in oysters
• decreased fecundity in freshwater snails
• egg abnormalities in freshwater snails

Tributyltin (TBT) has been used in antifouling paints

Background
Giusti et al. 2013
Higuera-Ruiz et al. 2000
Schulte-Oehlmann et al. 2004
Giusti et al. 2013
Aims of the study: to develop test methods and data analysis tools to evaluate toxic effects of TBT on a freshwater snail *Lymnaea stagnalis*

**Study objectives:**
- to assess relevance of the test protocol (OECD) for *L. stagnalis*
- to provide data suitable for mechanistic modelling of effects of EDCs
- to parameterize and calibrate a mechanistic model for individuals in control and contaminated conditions
Test animal
The pond snail *Lymnaea stagnalis*

- A freshwater hermaphroditic species, common in the northern hemisphere
- It has been proposed as a candidate species for OECD standard molluscs reproduction toxicity tests
Adult test setup (OECD standardisation)

Control treatment

![Image of Control treatment with 6x5 adult snails]

Exposure treatment

![Image of Exposure treatment with 6x5 adult snails]

Endpoints followed over time:
- **survival** – twice a week
- **shell length** – once a week
- **body dry mass** – end of the test
- **cumulative number of eggs** – collected twice a week

TBT exposures (ng/L):

- 325
- 650
- 1300
- 2600

Solvent: acetone 2 µl/L

Semi-static conditions. Renewal of the test medium: twice a week

Test duration: 28 days

Photoperiod: 14:10 h light:dark

Water temperature: 21°C

Feeding: *ad libitum* by fresh organic lettuce
Materials and methods

Juvenile test setup

Endpoints followed over time:
- **survival** – twice a week
- **shell length** – once a week
- **body dry mass** – end of the test
- **cumulative number of eggs** – collected twice a week

**Semi-static conditions. Renewal of the test medium: twice a week**

**Test duration: 35 days**

**Photoperiod: 14:10 h light:dark**

**Water temperature: 21°С**

**Feeding: *ad libitum* by fresh organic lettuce**

**TBT exposures (ng/L):**

- 11
- 24
- 53
- 117
- 258
- 587
- 907
- 1247
- 1995
- 2743

Solvent: acetone 20 µl/L
Mechanistic approach as a basis for modelling toxic effects

- Toxic effects modelled by implementing a toxicokinetic-toxicodynamic (TK-TD) model
- One-compartment first-order toxicokinetic model
- Accounting for concentration dilution by growth and by losses due to reproduction
- Toxicodynamics modelled using a process model for the organism

TK-TD model

**Input data**
- Survival
- Shell length
- Dry mass of egg clutches

**TBT exposures**

**Model outputs...**
- Survival
- Growth
- Reproduction

...predictions as mean values of responses
Conceptual approach for the “process model for organisms”

Dynamic Energy Budget (DEB) theory

The illustration taken from the previous presentation of Elke Zimmer
Process model for *L. stagnalis*

Simplified DEB model – **DEBkiss** (poster no. 18, *Jager et al.*)

- an explicit mass balance for an animal over its entire life cycle
- less parameters than standard DEB model
- convenient model for ecotox studies on invertebrates
Fitting the data for the control groups - juvenile and adult snails -
Fitting the data for the exposure groups - juvenile and adult snails -

Results and discussion
• Toxicokinetics differs between two tests - different concentrations of the carrier solvent used

Mode of action (DEB framework) of TBT:

Decrease in energy assimilation from food
Conclusions (I – model calibration):

• Using only snapshots from the life cycle we can successfully parameterise and calibrate the DEBkiss model
• Our test designs provided data needed for parameterisation and calibration of the model for TBT
• The model allowed estimation of biologically relevant model parameters for both control and exposed snails
• Model can be validated (independent data exist)
Conclusions (II - test design):

• An additional endpoint, the dry mass of the animal body, provides information on body shrinking
• Further recommendations for adapting test design for use of mechanistic models (like DEBkiss):
  - measuring the dry body mass (at several time points if possible)
  - both dry mass of egg clutches and egg numbers should be included in analyses
  - full life-cycle test
Thank you for your attention!

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References:


