Quantification of an Adverse Outcome Pathway by Bayesian network modelling: extrapolation from molecular events to demographic responses in *Lemna minor*

### Introduction
- **Adverse Outcome Pathways (AOP)** are useful for capturing toxicological knowledge to transparently link mechanistic data to apical toxicity endpoints, but their applicability for risk assessment are limited by the lack of quantitative models and assessment of uncertainties.
- **Quantitative AOPs (qAOP)** should define the **Key Event Relationships (KER)** sufficiently well to allow quantitative prediction of the probability and severity of the **Adverse Outcome (AO)** occurring for a given activation of the Molecular Initiating Event (MIE) (Conolly et al. 2017).
- We propose **Bayesian Networks (BN)** as a suitable approach for developing qAOPs.
- Here we present a BN model for quantification of a recently proposed AOP (Xie et al. 2018): linking the role of action of the respiratory and photosynthesis uncoupler 3,5-dichlorophenol (DCP) to an AO (reduced number of fronds) in the aquatic plant *Lemna minor* (Fig. 1).

### Data
- The data are obtained from a laboratory experiment (Xie et al. 2018):
  - *L. minor* was exposed to DCP in 8 concentrations (0.05, 0.1, 0.5, 1, 1.5, 2.5, 4, 8 mg/L) × 3 replicates.
- Measured response variables (day 7): OXPHOS (oxidative phosphorylation), ROS (reactive oxygen species), ETR (electron transfer rate), Fv/Fm (maximal yield of photosystem II), LPO (lipid peroxidation) and number of fronds (i.e. leaves, representing reproduction).
- The AOP (Fig. 1) is a network of three chains with the same chemical stressor (DCP) and AO (fronds number). OXPHOS and ROS are both defined as MIEs; ROS is initiated at a higher concentration.

### Methods
- **Statistical modelling.** For each Key Event Relationship (KER),
  - A suitable dose-response model was selected based on exploratory data analysis (generalized additive models) and the model selection tool in r (Ritz et al. 2015).
  - The fitted curve with standard errors (Fig. 2) was used to simulate new values (n = 10000) along the axis, for generating probability distributions for the CPTs (Table 1).
- **BN modelling.**
  - All AOP components were defined as nodes with discrete states (intervals) (Fig. 3).
  - The KERs were defined by **conditional probability tables (CPTs)** (Tables 1-2), which determine the probability distribution of a node given probability distribution of its parent node(s).
  - CPTs can be quantified by different approaches, such as counts of observations, probability, or expert judgment.
  - The BN was run by changing the DCP concentration and inspecting the changes in all subsequent nodes (Fig. 3), and backwards from a selected **Adverse Outcome state** (Fig. 4).

### Results
- **AOP model (Fig. 3)** predicted responses of all MIEs, KEs and AOs to increasing chemical stressor concentration in accordance with the conceptual model (Fig. 1, arrows up/down).
- Increasing DCP up to 3 mg/L resulted in decreased fronds no. in all three pathways.
- The pathway OXPHOS → ETR → Fronds no. was the most sensitive to changes in the chemical stressor.
- The low sensitivity of the pathway ROS → LPO → Fronds no. was the most sensitive to changes in the chemical stressor.
- The quantification of Key Event Relationships will be improved by further testing of suitable stressors based on CPTs (here: fronds number >100) to predict the e.g. the required state of the chemical stressor.
- BN models quantified by different approaches (Table 1) showed different performance:
  - CPTs based on count of observations (Table 1a) gave more accurate predictions at high and low stressor concentrations.
  - CPTs based on statistical models (Table 1b) gave more accurate predictions at intermediate stressor concentrations.
- Example of diagnostic use of the BN (Fig. 4): the best outcome state (fronds no. >100) requires a 89% probability of DCP concentration <1.25 mg/L.

### Further developments
- The quantification of Key Event Relationships will be improved by further testing of suitable dose-response models, and by using Bayesian statistics (R package “bayesglm”) for better simulation of variability (Fig. 2).
- The Adverse Outcome will be extended to the population level (e.g. intrinsic population growth), which may have higher regulatory relevance.
- The exposure to the chemical stressor will be linked to an Aggregate Exposure Pathway also modelled as a BN.
- The model can be further extended to a full Source-To-Outcome Pathway.

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### References

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**Figure 2.** Quantification of the Key Event Relationships and their uncertainty by regression models. Red dots = observations; blue curve = fitted values; grey grid lines = intervals of the probability distribution of a node given probability distribution of its parent node(s). The colour scale indicates the proportion (green = 0%, red = 100%), which correspond to probabilities in the CPT. The CPT based on statistical models (Fig. 2a) was used to simulate new values (n = 10000) output of the statistical model (Fig. 2d). The number of observed or simulated values in each grid cell is used to obtain probabilities for the CPTs (Table 1a and b), respectively.

**Table 1.** Example of a conditional probability table (CPT) for ROS dependent on DCP, based on the estimated standard error, grey grid lines = intervals of the nodes (Fig. 2). The number of observed or simulated values in each grid cell is used to obtain probabilities for the CPTs (Table 1a and b), respectively.

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**Figure 3.** BN representing a quantitative AOP network for effects of DCP on reproduction of *Lemna minor*, quantified by statistical models (cf. Table 1b). (Nodes names such as “fronds number (ETR)” means “fronds number predicted from ETR”). The shifts in probability distributions as the DCP concentrations increases from low (a) to high (b), corresponding to the qualitative changes indicated by the conceptual model (Fig. 1).

**Figure 4.** Diagnostic use of the AOP-BN: running the model backwards from a selected state of the Adverse Outcome (here: fronds number >100) to predict the e.g. the required state of the chemical stressor.