Quantification of an AOP network for UV radiation by exploratory data analysis and probabilistic modelling

S. Jannicke Moe[1], Knut Erik Tollefsen[1,2,3], Li Xie[1,3], You Song[1,3]

[1]Norwegian Institute for Water Research (NIVA), Oslo, Norway
[2]Norwegian University of Life Sciences (NMBU), Ås, Norway
[3]Centre for Environmental Radioactivity (CERAD), Ås, Norway
email: jmo@niva.no


Photo: You Song

Highlights

★ A proposed **Adverse Outcome Pathway (AOP) network** describes adverse effects of **UV-B radiation** on *Daphnia magna*

★ We quantify all **Key Event Relationships (KERs)** by non-linear regression methods

★ We use a **Baysian Network (BN)** model for linking all Key Events (KEs) by **conditional probability distributions**

★ The BN can be run forwards and **backwards**

Background

- **Adverse Outcome Pathways (AOPs)** capture the biological complexity in causal networks and mechanistically link toxic effects at increasing levels of biological organization to apical endpoints with relevance for risk assessment.
- **Quantitative AOPs (qAOP)** should quantify the **Key Event Relationships (KER)** to allow prediction of the probability and severity of the **Adverse Outcome (AO)** occurring for a given state of the **Molecular Initiating Event (MIE)** or a **Key Event (KE)** (Conolly et al. 2017).
- The proposed AOP network integrates experimental data from **genetic biomarkers** with physiological and **demographic endpoints**
- We propose **Bayesian Networks (BN)** as an approach for quantifying and assessing the structure of AOP networks based on limited data.

Data

The data are obtained from a lab experiment (Song et al. unpubl.):

- *Daphnia* was exposed UV-B radiation in 6 dose-rates: 0, 0.0008, 0.05, 0.1, 0.2, 0.4 w/m^2.
- Each response variable (Fig. 1) was measured in 3-6 samples.
- The scale of each response variable is relative to the control treatment (1 = equal to contol).
- For each KER, all possible combinations of pseudoreplicates were used as observations.
- The proposed AOP (Fig. 1) is a network of several possible pathways with the same stressor, MIE and AO.

Approach

Statistical modelling. For each Key Event Relationship (KER), we used:

1. a regression tree model to estimate breaks along the x-axis (Fig. 2);
2. a generalised Additive Model (GAM) to estimate the strength and the shape, allowing for non-linear and flexible dose-response or **response-response relationships** (Fig. 2, Table 1);
3. a similar parametric dose-response model fitted by the R tool *drc* (Ritz et al. 2015) (Fig. 3);
4. the fitted curve with standard errors (Fig. 3) to simulate new values (n = 10000) along the x-axis.

Bayesian Network modelling.

5. All AOP components were defined as **nodes** with discrete intervals.
6. The KERs were defined by **conditional probability tables (CPTs)**, (Table 2), with probability distributions obtained from Step 4.
7. Model running: The BN uses CPTs to calculate the probability distribution of a child node based on the probability distribution of its parent nodes (Fig. 4a, b) – or vice versa (backwards; Fig. 4c, d).

Conceptual model

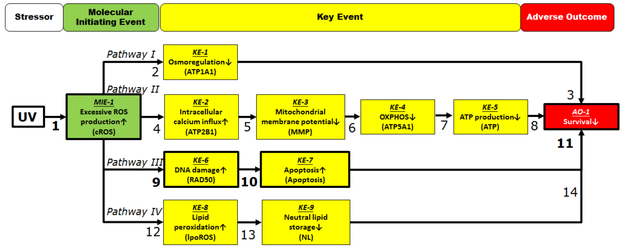
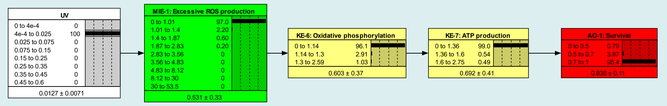


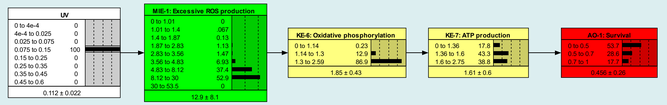
Figure 1. Proposed AOP network for effects of UV on *Daphnia magna*. Each Key Event Relationship (KER) is represented by a numbered arrow (see Table 1). The quantification of KERs is illustrated (Figs. 2, 3, 5) for the most significant Pathway (III): UV → Excessive ROS production (MIE-1) → DNA damage (KE-6) → Apoptosis (KE-7) → Survival (AO-1).

Examples of BN model run from scenarios of...

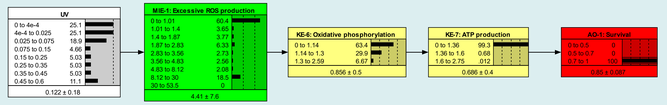
(a) Low stressor level (UV dose)



(b) High stressor level (UV dose)



(c) Least adverse outcome (high survival)



(d) Most adverse outcome (low survival)

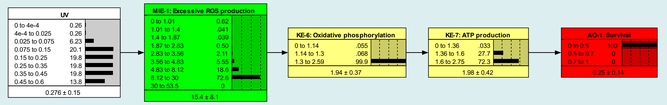


Figure 4. BN model representing Pathway III of the draft qAOP network. Arrows represent CPTs (see Table 2). The examples show the BN model run forwards from low or high stressor level (**a**, **b**) and backwards from low and high AO level (**c**, **d**).

Table 1. Assessment of the proposed KERs (Fig. 1) based on GAM analysis (Fig. 2). The **deviance** explained by a GAM quantifies the strength of the KER. The estimated **degrees of freedom** (df) quantifies the degree of non-linearity of a KER. A positive (vs. negative) **sign** of KER means that its wo Key Events are positively (vs. negatively) correlated.

KER no.	KER label	p-value	Deviance explained	Estimated df	Expected sign of KER	Estimated sign of KER
1	UV → MIE1	<0.001	38 %	2.9	Positive	Convex
2	MIE1 → KE1	<0.001	48 %	4.9	Negative	Convex
3	KE1 → AO1	<0.001	8 %	4.5	Positive	Convex
4	MIE1 → KE2	<0.001	26 %	3.9	Positive	Convex
5	KE2 → KE3	<0.001	24 %	4.6	Negative	Concave
6	KE3 → KE4	<0.001	12 %	1.3	Positive	Positive
7	KE4 → KE5	<0.001	39 %	3.5	Positive	Convex
8	KE5 → AO1	<0.001	53 %	5.0	Positive	Positive
9	MIE1 → KE6	<0.001	31 %	4.9	Positive	Positive
10	KE6 → KE7	<0.001	46 %	5.0	Positive	Positive
11	KE7 → AO1	<0.001	31 %	4.8	Negative	Negative
12	MIE1 → KE8	<0.001	27 %	3.1	Positive	Positive
13	KE8 → KE9	<0.001	19 %	4.4	Negative	Convex
14	KE9 → AO1	<0.001	34 %	4.3	Positive	Positive

Results

- The estimated sign of the KERs (Fig. 2) were mostly consistent with the expectations (Table 1).
- Most of the estimated KERs showed strong non-linearity (Fig. 2-3, Table 1).
- GAMs explained 31-46% of the deviance for KERs in Pathway III (Table 1).
- Increasing UV dose from low to intermediate resulted in higher probability of low survival (Fig. 4a, b).
- Running the BN backwards from "high survival" resulted in a bimodal distribution of UV dose, reflecting the convex shape of KER no. 1 (UV → MIE; Fig. 4c).
- For "low survival", the BN predictes 93% probability UV dose exceeding 0.075 w/m^2 (Fig. 4d).

Future model development

- Improve the quantification of the KERs by further evaluation of suitable **dose-response models**
- Use **Bayesian regression models** for better simulation of new values (Fig. 3).
- Address **pseudoreplicates** in response-response relationships
- Use **sensitivity analysis** for ranking the pathways according to their influence on the adverse outcomes
- Optimize the **weighting** of the four pathways for the joint AO
- Extend the AO to **population-level endpoints** level with higher regulatory relevance, e.g. intrinsic population growth rate

Figure 2. The estimated shape and strength (% deviance explained) of KERs in Pathway III. Red dots = observations; black curves = regression curve; grey shade = confidence intervals.
Break points (non-linearities) estimated along an x-axis are shown as blue vertical lines, and as green horizontal lines when this variable is used as y-axis. Wider lines show more significant break points.

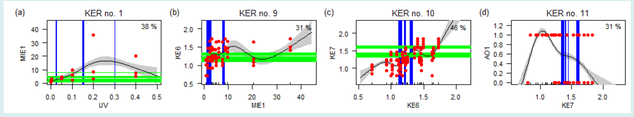


Figure 3. Quantification of the selected KERs (from Fig. 2) and their uncertainty by dose)-response regression.
Red dots = observations; red curves = regression curve;
grey shade = confidence intervals; black dots = simulated values.
Grey grid lines = selected break points from Fig. 2.

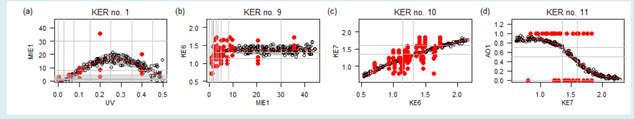


Table 2. Conditional probability tables for quantifying Key Event Relationships. The probabilities are counts of simulated observations per grid cell in Fig. 3.

(a) KER no.1:
UV → Excessive ROS production (cROS)

UV / MIE-1	[0, 0.0004]	[0.0004, 0.0254]	[0.0254, 0.075]	[0.075, 0.15]	[0.15, 0.25]	[0.25, 0.35]	[0.35, 0.45]	[0.45, 0.6]
[30, 53.5]	0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %
[8.12, 30)	0 %	0 %	0 %	53 %	100 %	100 %	84 %	30 %
[4.83, 8.12)	0 %	0 %	9 %	37 %	0 %	0 %	13 %	24 %
[3.56, 4.83)	0 %	0 %	13 %	7 %	0 %	0 %	2 %	11 %
[2.83, 3.56)	0 %	0 %	13 %	1 %	0 %	0 %	1 %	4 %
[1.87, 2.83)	0 %	0 %	21 %	1 %	0 %	0 %	0 %	5 %
[1.4, 1.87)	0 %	1 %	11 %	0 %	0 %	0 %	0 %	3 %
[1.01, 1.4)	0 %	2 %	10 %	0 %	0 %	0 %	0 %	2 %
[0, 1.01)	100 %	97 %	22 %	0 %	0 %	0 %	1 %	21 %
Count	8	500	992	1500	2000	2000	2000	1000

(b) KER no.9:
cROS → DNA damage (RAD50)

MIE-1 / KE-6	[0, 1.01)	[1.01, 1.4)	[1.4, 1.87)	[1.87, 2.83)	[2.83, 3.56)	[3.56, 4.83)	[4.83, 8.12)	[8.12, 30)	[30, 53.5]
[1.3, 2.59)	0 %	0 %	0 %	7 %	47 %	74 %	96 %	85 %	82 %
[1.14, 1.3)	1 %	13 %	77 %	92 %	52 %	26 %	4 %	15 %	18 %
[0, 1.14)	99 %	88 %	23 %	0 %	1 %	0 %	0 %	0 %	0 %
Count	95	88	108	218	167	287	749	4972	3316

(c) KER no.10:
RAD50 → Apoptosis

KE-6 / KE-7	[0, 1.12)	[1.12, 1.16)	[1.16, 1.3)
[1.6, 2.75]	0 %	0 %	45 %
[1.36, 1.6)	0 %	0 %	50 %
[0, 1.36)	100 %	100 %	5 %
Count	3746	1014	5240

(d) KER no.11:
Apoptosis → Survival

KE-7 / AO-1	[0, 1.36)	[1.36, 1.6)	[1.6, 2.75]
[0.7, 1]	96 %	1 %	0 %
[0.5, 0.7)	4 %	65 %	0 %
[0, 0.5)	0 %	34 %	100 %
Count	4469	1415	4116

References

Conolly RB, Ankley GT, Cheng W, Mayo ML, Miller DH, Perkins EJ, Villeneuve DL, Watanabe KH. 2017. Quantitative adverse outcome pathways and their application to predictive toxicology. *Environtal Science and Technology* 51:4661–4672.

Ritz C, Baty F, Streibig JC, Gerhard D. 2015. Dose-response analysis using R. *PLOS ONE* 10: e0146021.

Acknowledgements

This work was funded by the Research Council of Norway through the Centre for Environmental Radioactivity (Project No. 223268/F50, www.niva.no/cerad), and by NIVA's Computational Toxicology Program (www.niva.no/nctp).

This poster

NIVA's Section for Ecotoxicology and Risk Assessment