Integrating Point of Departure and Structural Equation Modelling to AOP Development_ A Case Study of Diuron Toxicity in Microalgae

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Introduction and objective

Adverse Outcome Pathways (AOPs) and their quantitative extensions (qAOPs) provide structured frameworks to understand toxicological mechanisms to support risk assessment. However, quantitative data for key event relationships (KERs) are often limited. This study used analysis of multiple points of departure (PoD), including No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), Benchmark Dose (BMD) and half-maximal effective concentration (EC50), combined with structural equation modelling (SEM) to verify and quantify the causal relationships within an AOP network. A case study was developed to illustrate the approach involving data generation (*Chlamydomonas reinhardtii* exposed to Diuron), analysis of dose-response data for PSII inhibition, photosynthesis, mitochondrial membrane potential (MMP), ATP content, and growth at different exposure durations.

Tiered approach to develop a quantitative AOP

AOP construction and weight of evidence assessment

Conceptual AOP construction

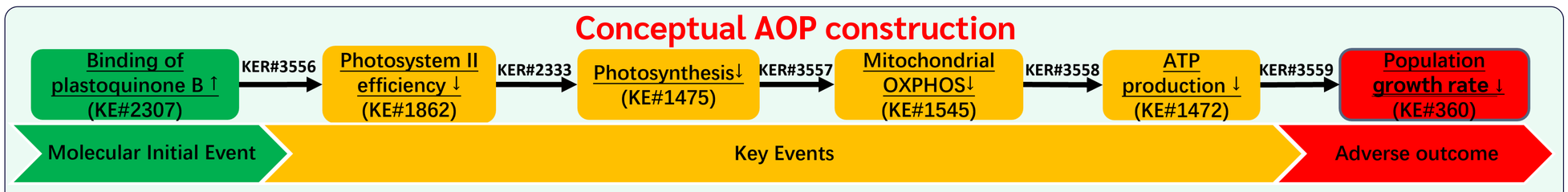


Fig.1 Binding of plastoquinone B leading to decrease, population growth rate via decrease, photosystem II efficiency (AOP#567, AOPwiki)

Weight of evidence assessment

AI-based literature review

AOP-helpFinder

AOP-helpFinder 3.0

S^2CIE

AOP-BOT

Scoping review

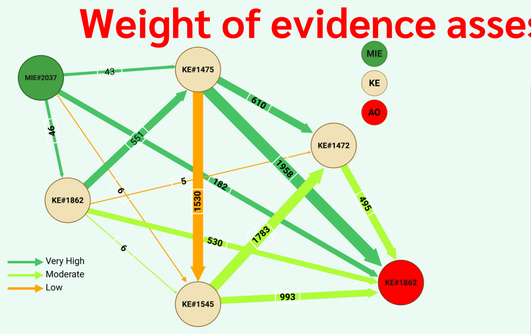


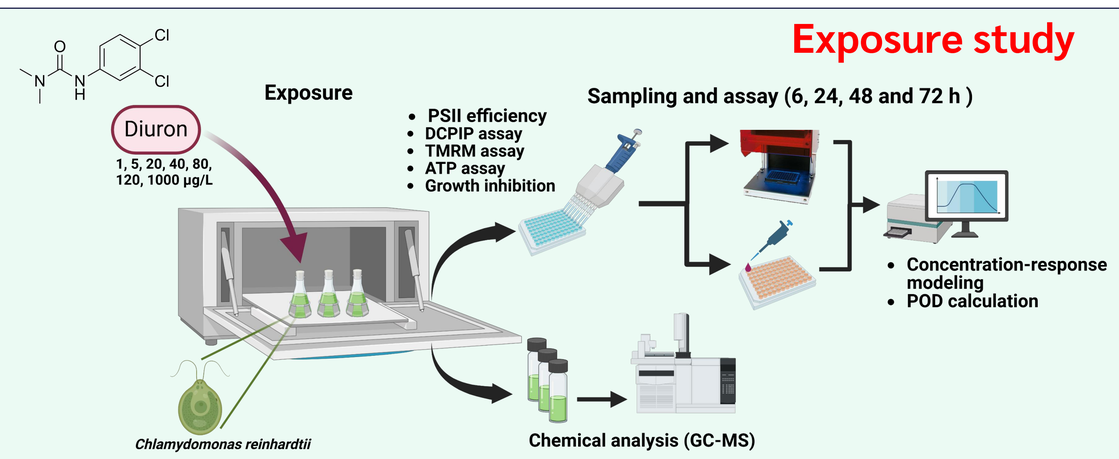
Fig.2 Count Score (CS) and confidence Score of publications associated with KER evaluated by AOP-helpFinder

Table 1. Weight of evidence (WOE) assessment of key events relationships

KER #	Title	Essentiality	Biological plausibility	Empirical support	Quantitative understanding	Overall WoE
3556	Binding of plastoquinone B leads to Decrease in Photosystem II efficiency	High	High	High	High	High
2333	Decrease in PSII efficiency leads to Decrease in Photosynthesis	High	High	High	High	High
3557	Decrease in Photosynthesis causes Decrease in Mitochondrial OXPHOS	moderate	Moderate	Moderate	High	Moderate
3558	Decrease in Mitochondrial OXPHOS leads to Decrease in ATP production	High	High	High	High	High
3559	Decrease in ATP production leads to Decrease in Population growth rate	moderate	High	High	High	Moderate

Concentration–response data and PoD analysis

Exposure study



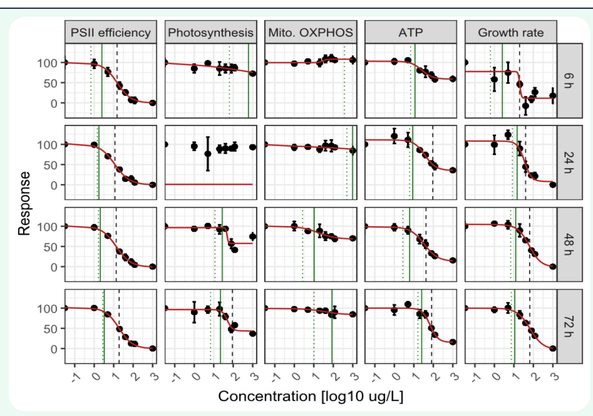


Fig 3. Concentration-response relationship (CRR) and point of departure (POD) analysis for the different endpoints after exposure.

Quantitative evaluation of KER using SEM

Structural equation modeling (SEM)

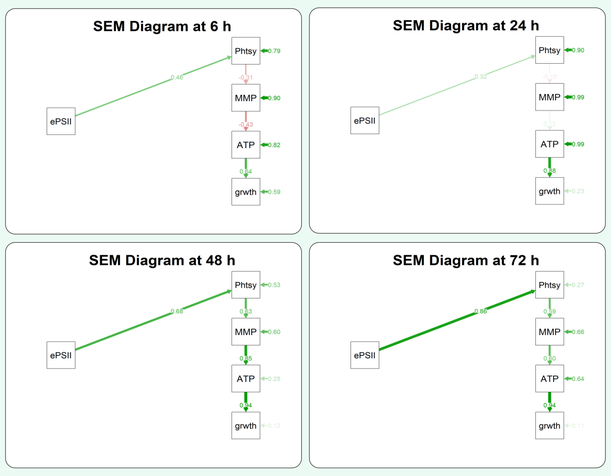


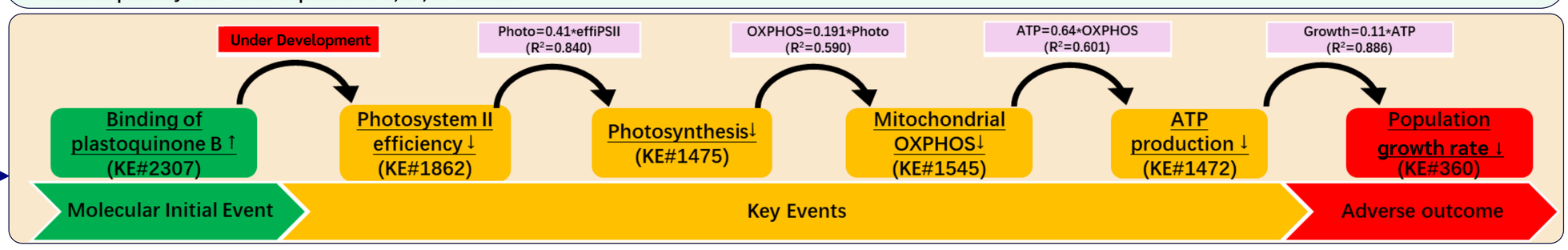
Fig 4. **Structural equation modeling visualization of the adverse outcome pathway (AOP) after exposure for 6, 24, 48 and 72h**

Table 3. Four best-fit model evaluating temporal responses from Diuron-induced PSII inhibition to growth on Chlamydomonas reinhardtii.

Chain	*Effi*PSII	Photo	MMP	ATP	Growth	P	CFI	RMSEA	SRMR
1	24h	72h	72h	72h	72h	0.02	0.862	0.45	0.077
2	6h	72h	72h	72h	72h	0.02	0.873	0.44	0.077
3	72h	72h	72h	72h	72h	0.02	0.872	0.44	0.078
4	48h	72h	72h	72h	72h	0.02	0.866	0.45	0.080

EffiPSII = effective Photosystem II efficiency; Photo = Photosynthesis; MMP = mitochondrial membrane potential; ATP = adenosine triphosphate; Growth = growth; P = p-value; CFI = Comparative Fit Index; RMSEA = Root Mean Square Error of Approximation; SRMR = Standardized Root Mean Square Residual.

Quantitative AOP Development (In Progress)



Conclusion

This work proposes a hybrid framework combining Concentration–response relationship (CRR)-, PoD-based analysis and SEM to consolidate qAOP development using diuron toxicity in *Chlamydomonas reinhardtii* as an example. This approach enhances the mechanistic understanding and strengthens ecological risk assessment by offering clearer benchmarks and insights into exposure-duration dependencies for individual responses in qAOPs.

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