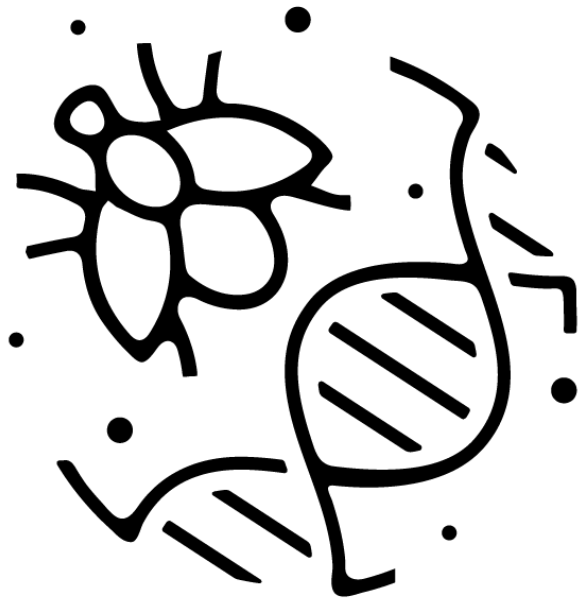
May 19th , 2026

From Theory to Practice: Employing EcR Agonism AOP for *In Vitro* Screening Assay



Rebeka Darmati

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WAGENINGEN UNIVERSITY & RESEARCH

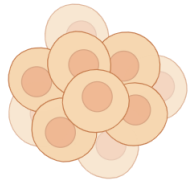
Unilever

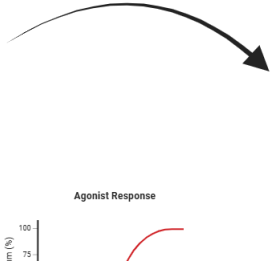
tox | Division of Toxicology Wageningen University

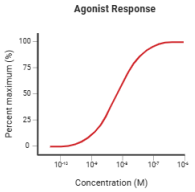
CARDIFF UNIVERSITY

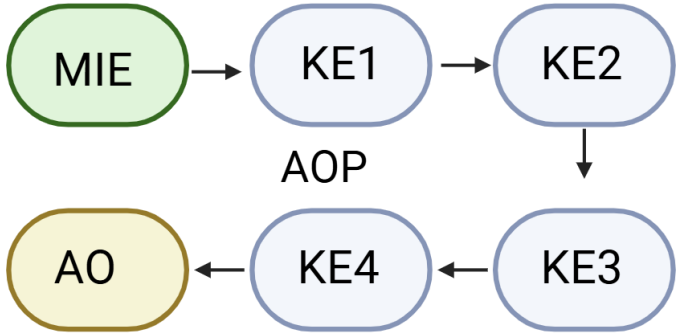
Aim of the project

- Develop an *in vitro* screening assay for invertebrate specific traits

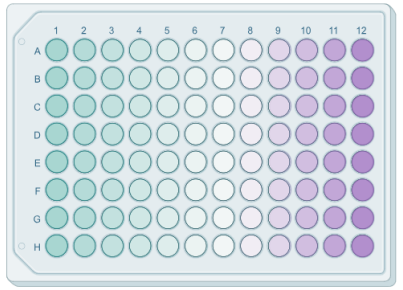






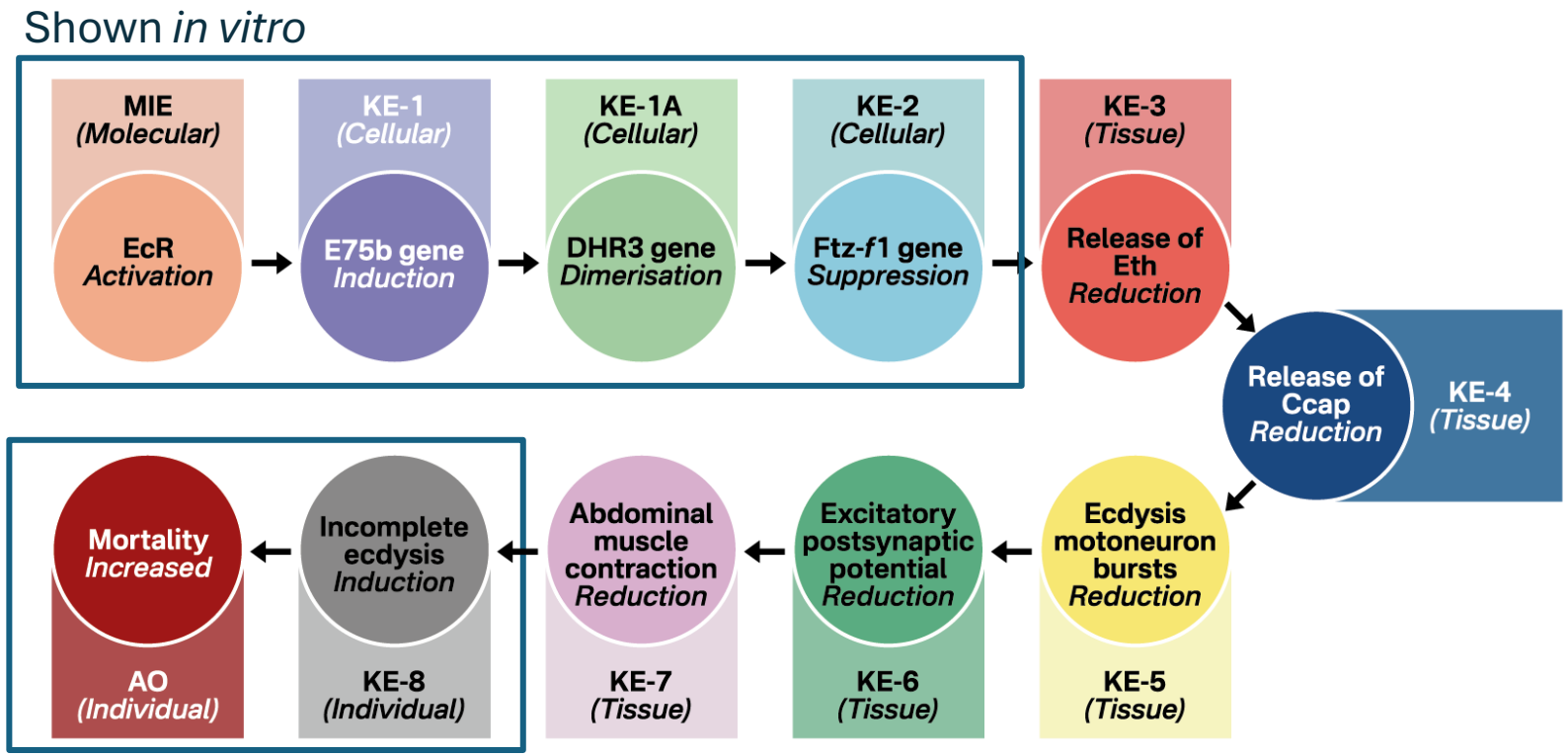


In vitro screening assay



AOP4: Ecdysone receptor agonism leading to incomplete ecdysis associated mortality

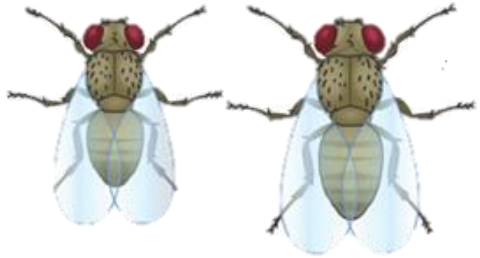
- Ecdysis
  - underlies development
- Controlled by Ecdysone
- **EcR Agonism** as a Molecular Initiating Event



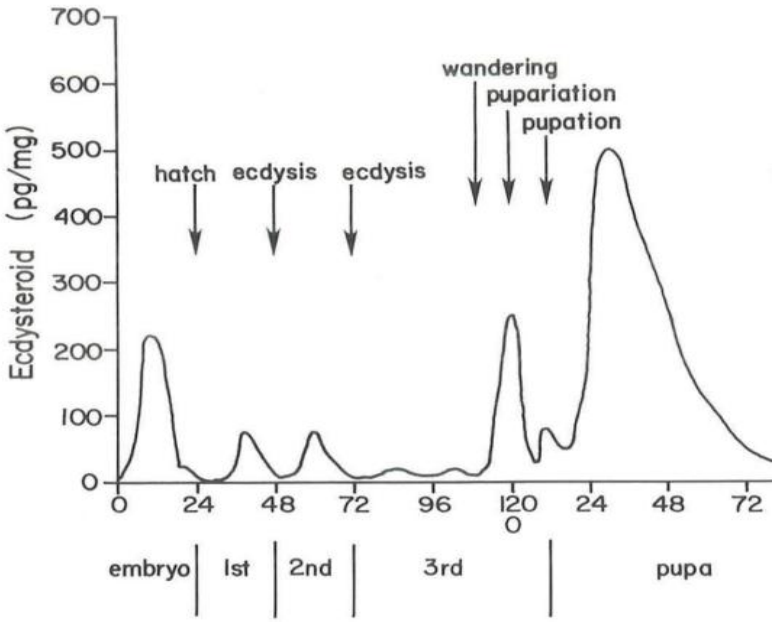
Adapted from Song et al., (2017)

Drosophila melanogaster

- Developmental stages
- Ecdysone released in pulses
- Ecdysis – genetic cascade



Adult



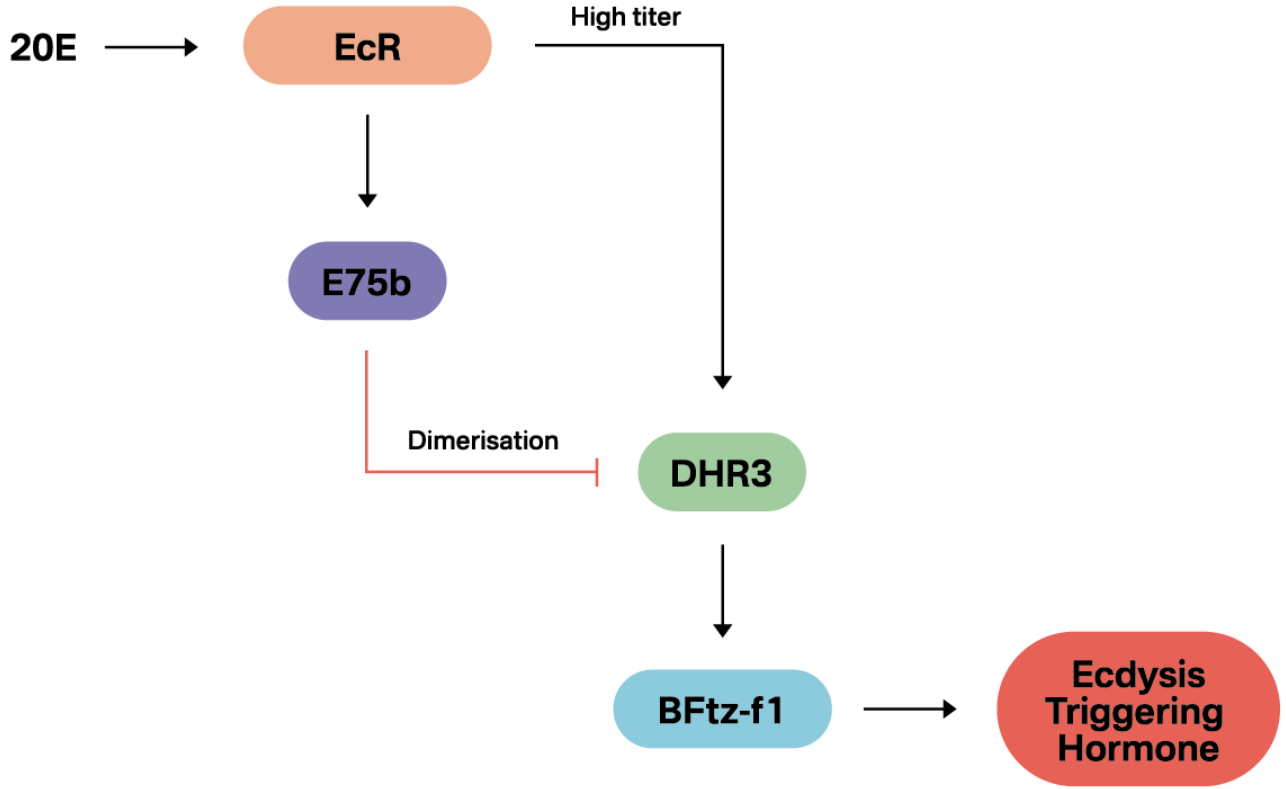
Riddiford, (1993)

Adapted from FlyMove

Ecdysis genetic cascade

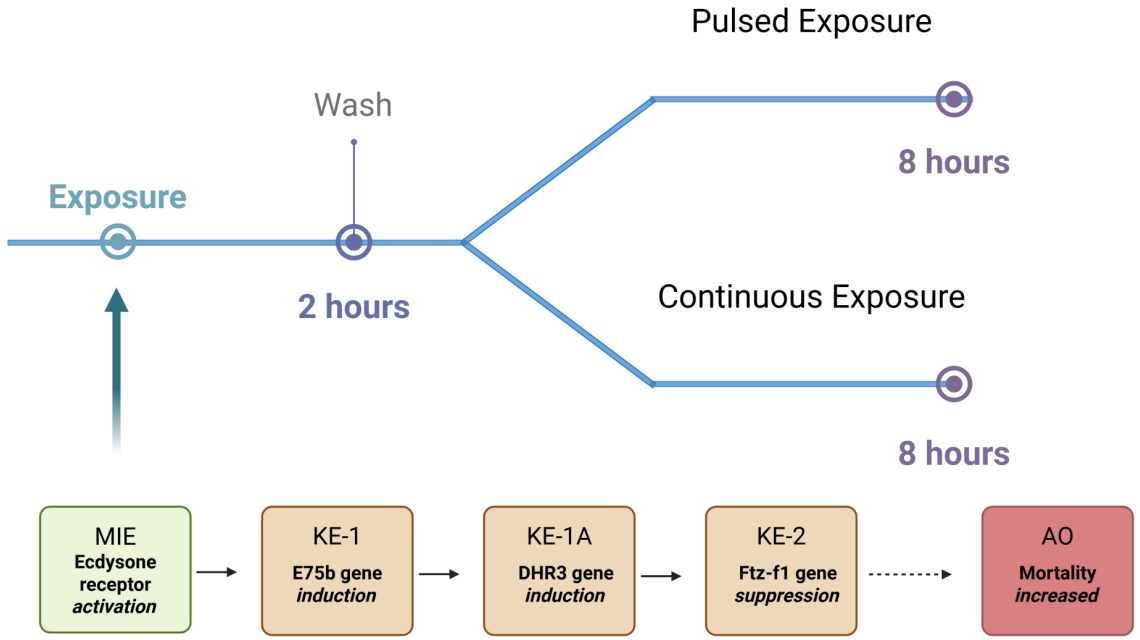
- Cascade of transcription factors
- Conserved pathway

- Continuous EcR agonism can block the expression of Ftz-f1
  - **Incomplete ecdysis**

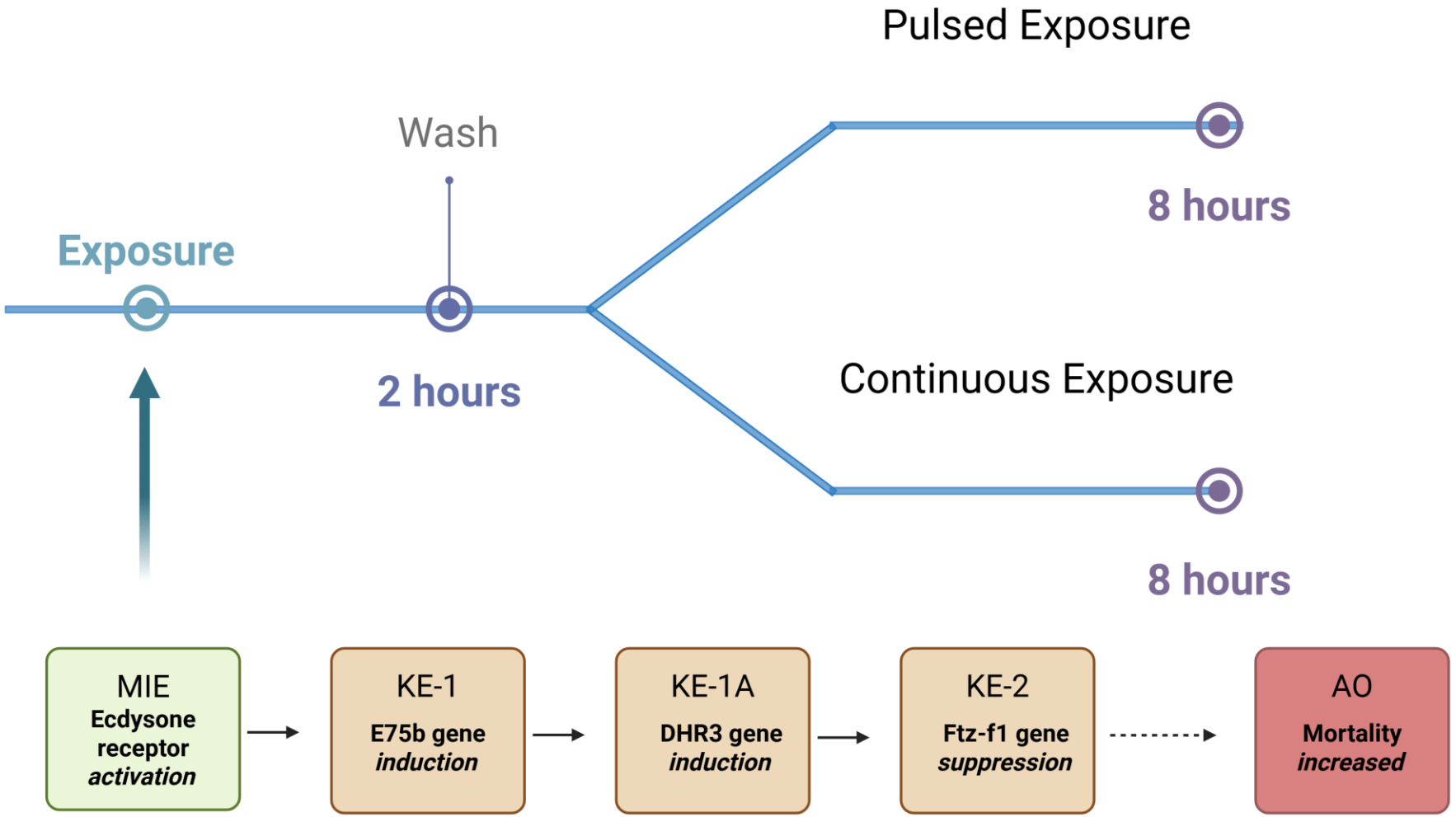


Proof of concept for the initial KEs of the EcR agonism AOP *in vitro*

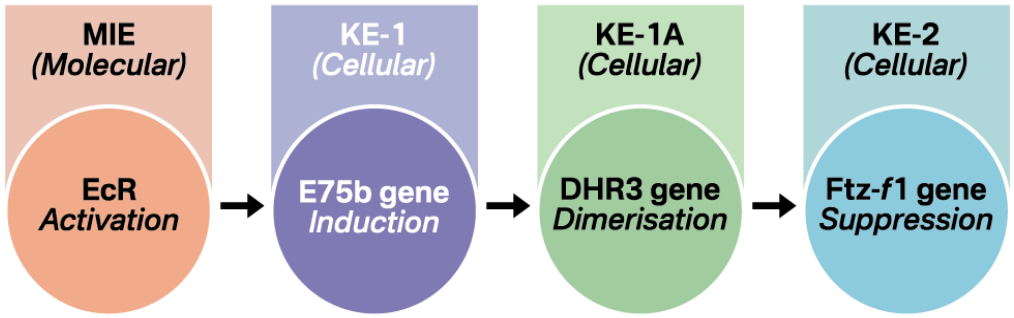
- Determine the ability of S2 cell line to portray the **initial KEs of the EcR agonism AOP**
- Exposure to Ecdysone
  - Temporal expression profiling
  - Concentration dependant expression



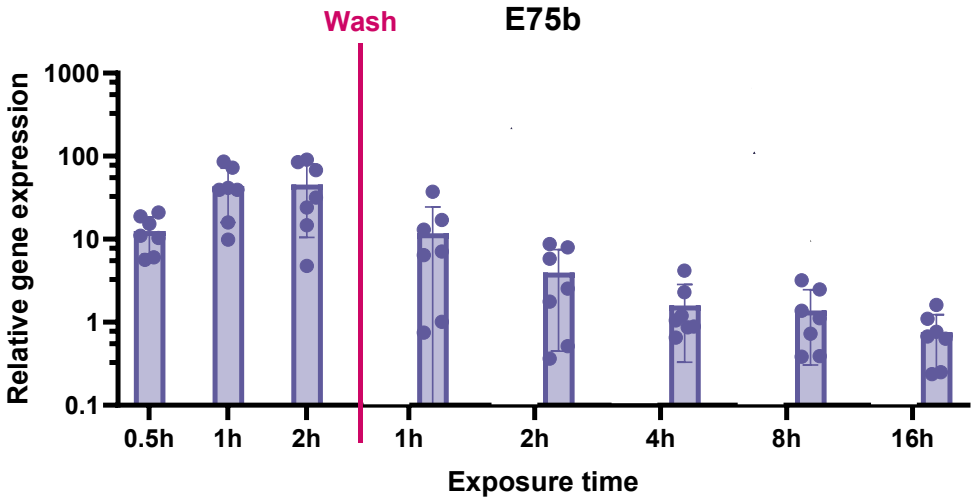
Proof of concept for the initial KEs of the EcR agonism AOP *in vitro*

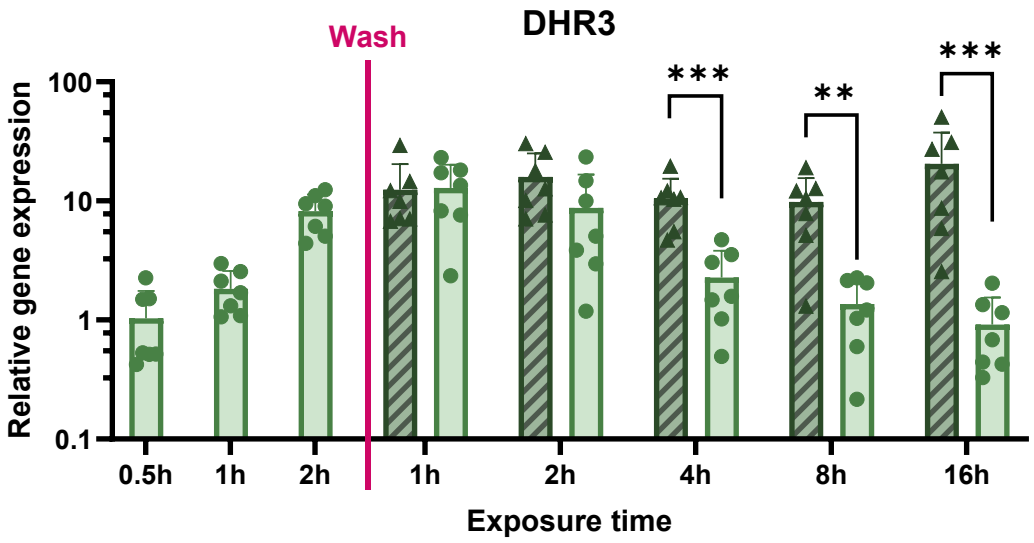


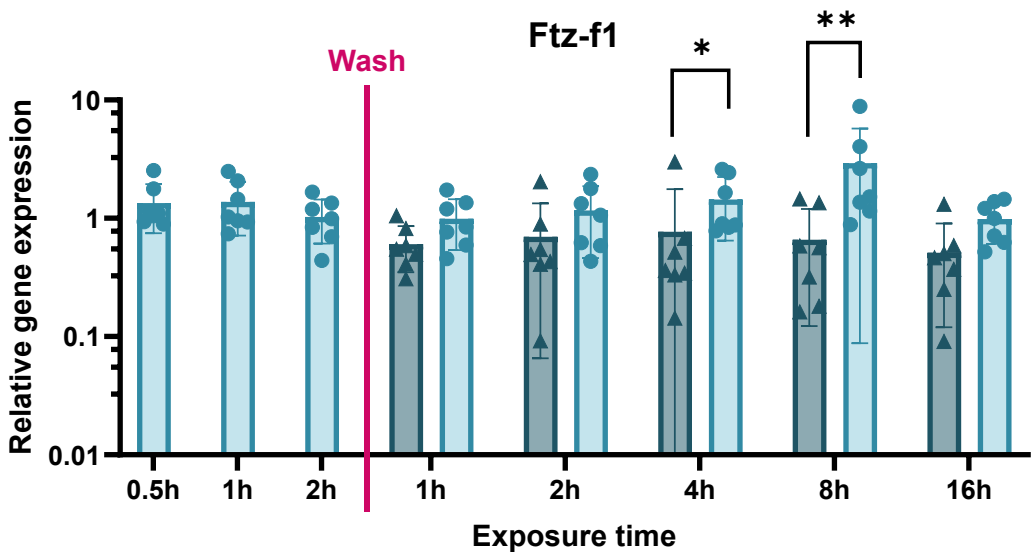
S2 cells mimic the initial KEs of the AOP



- ▲ Continuous
- ● Pulsed



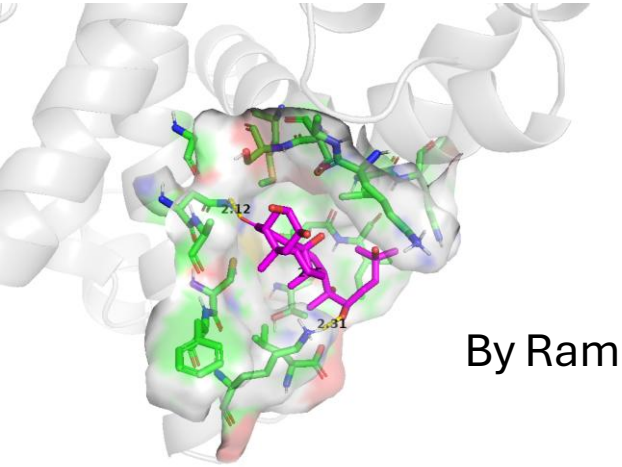




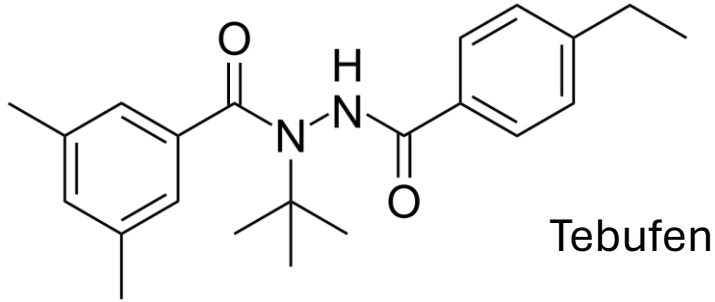
Exposure to
0.1 µM of 20E

In silico assessment

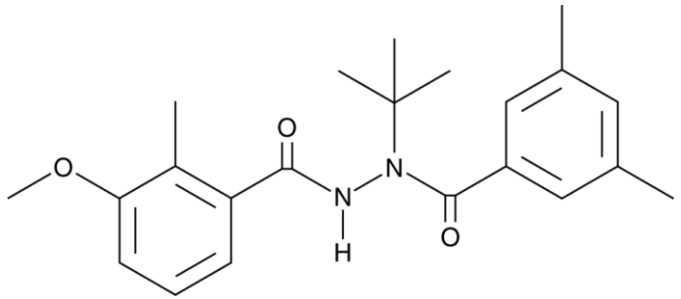
- Ecdysone receptor agonists:
- *In silico* assessment of molecular docking binding affinities



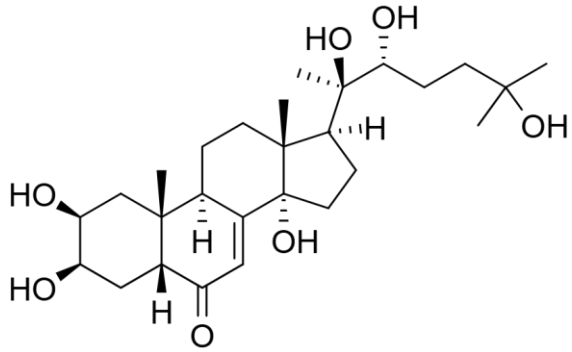
By Rama Krishnan



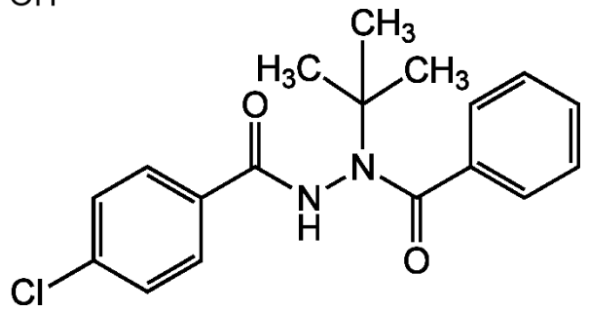
Tebufenozide



Methoxyfenozide

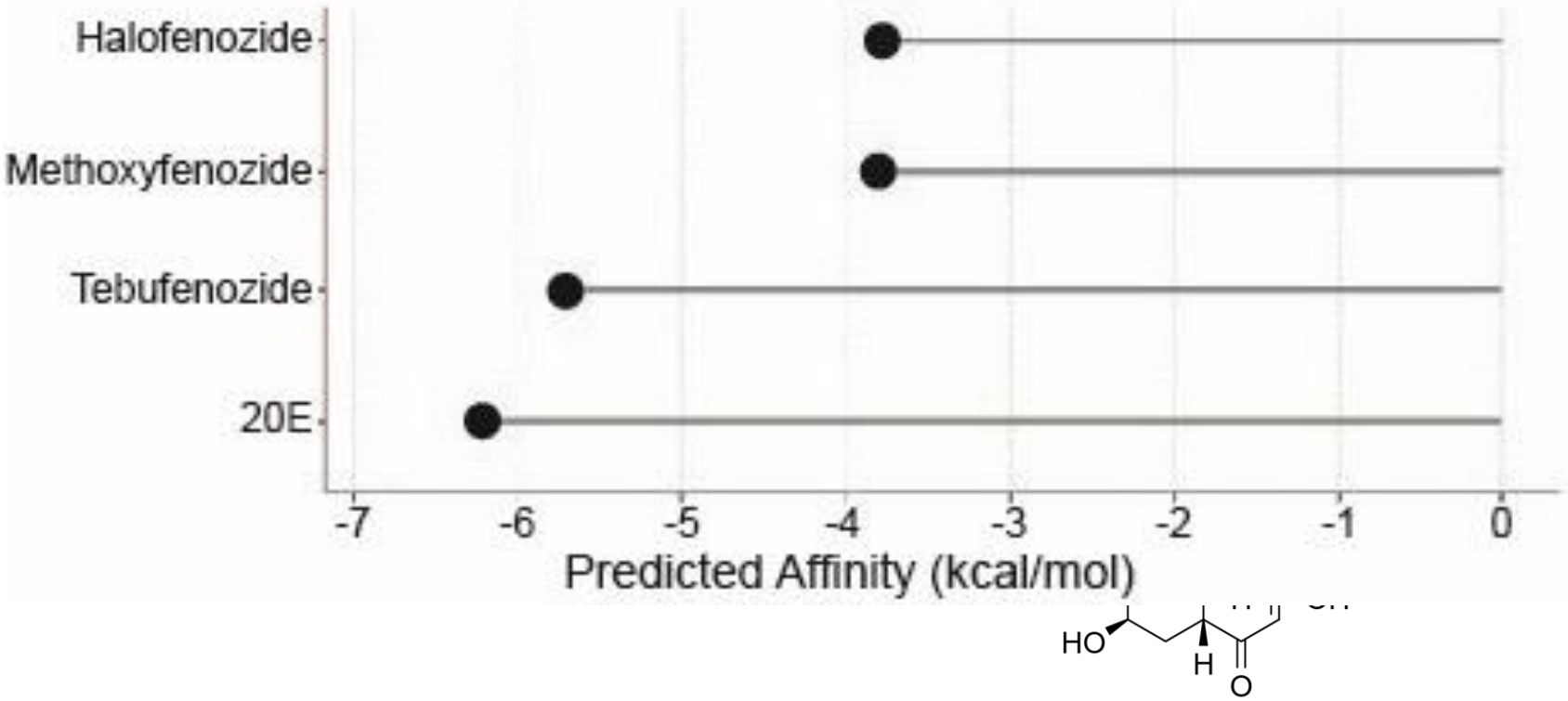


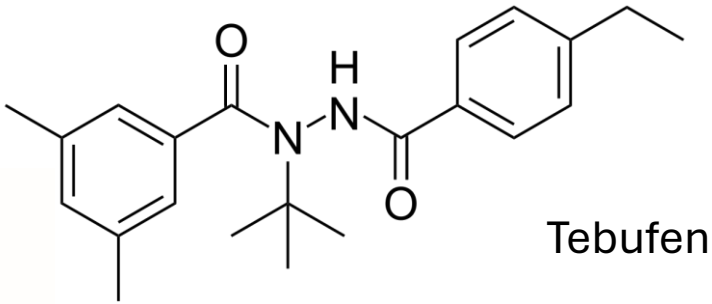
20- Hydroxyecdysone



Halofenozide

In silico assessment





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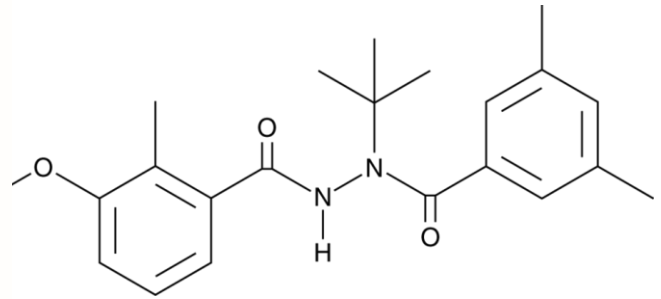

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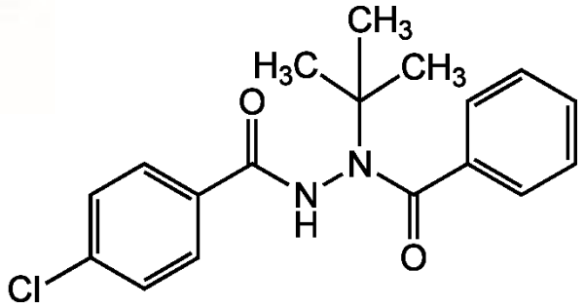
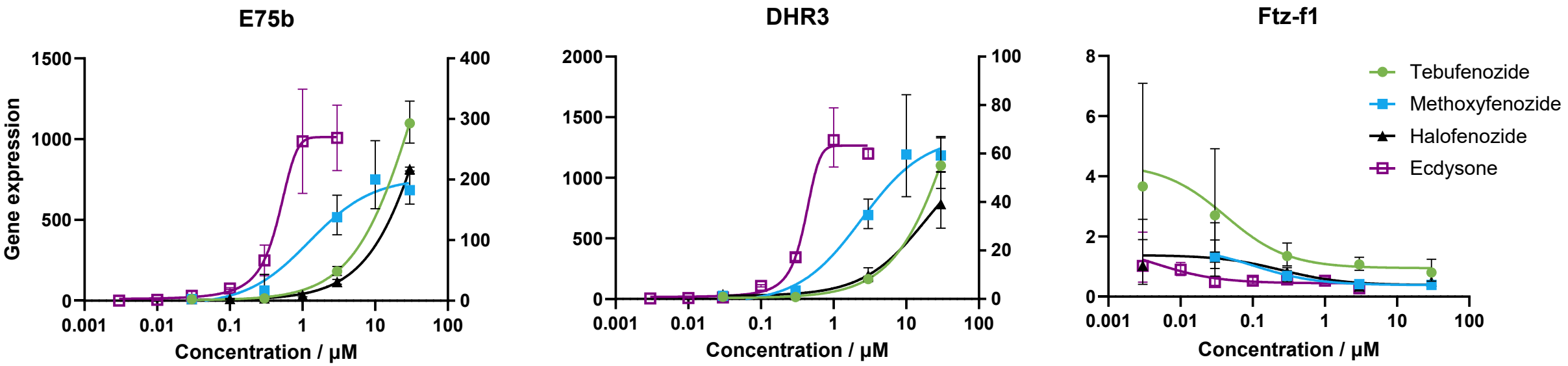
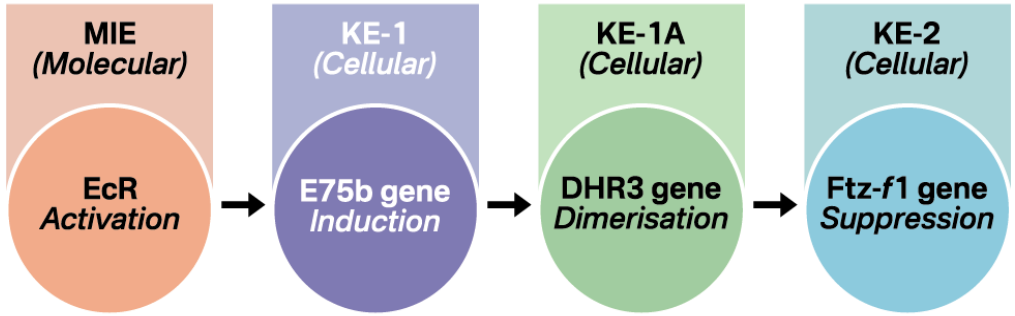


Halofenozide

In vitro assessment



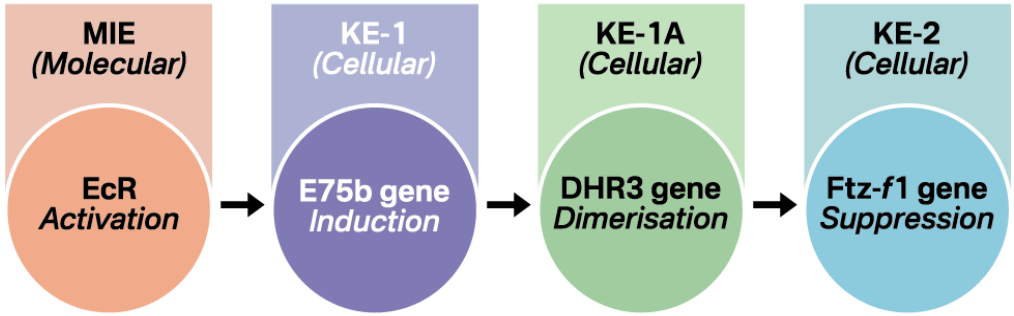


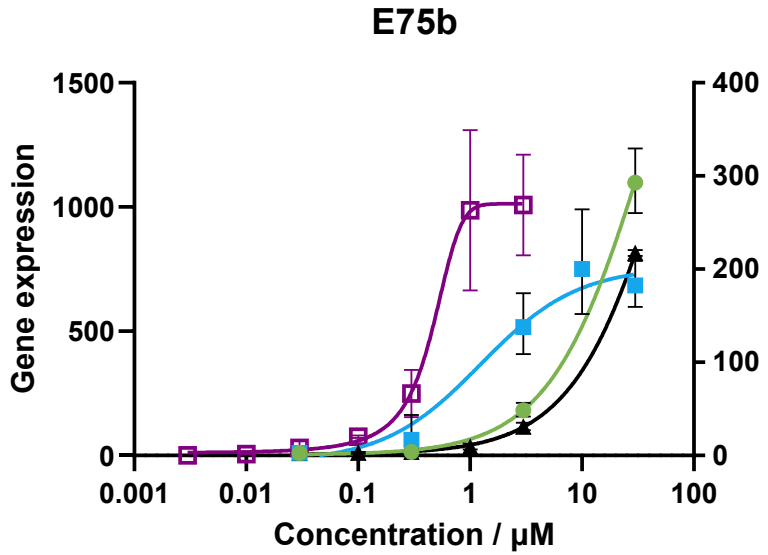
Key points

S2 cells can portray the initial KEs of the EcR agonism AOP

Employing S2 cells for an *in vitro* assay supports early development of chemicals

In vivo studies for confirmation of the AO are underway





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