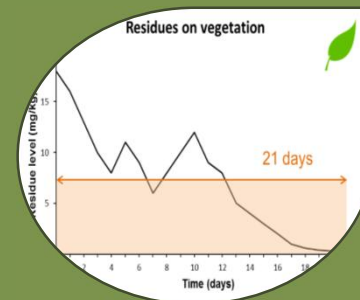


# 3rd Virtual Workshop on the revised EFSA Birds and Mammals Guidance Document

Background, Regulatory hurdles, Ambiguities, Lines of evidence, Modelling - How best to approach the new fTWA assessment.



📅 April 28, 2026  
🕒 10 am – 3 pm CET

*Thomas Martin* - RIFCON  
**Topic 3b:** modelling  
lines of evidence



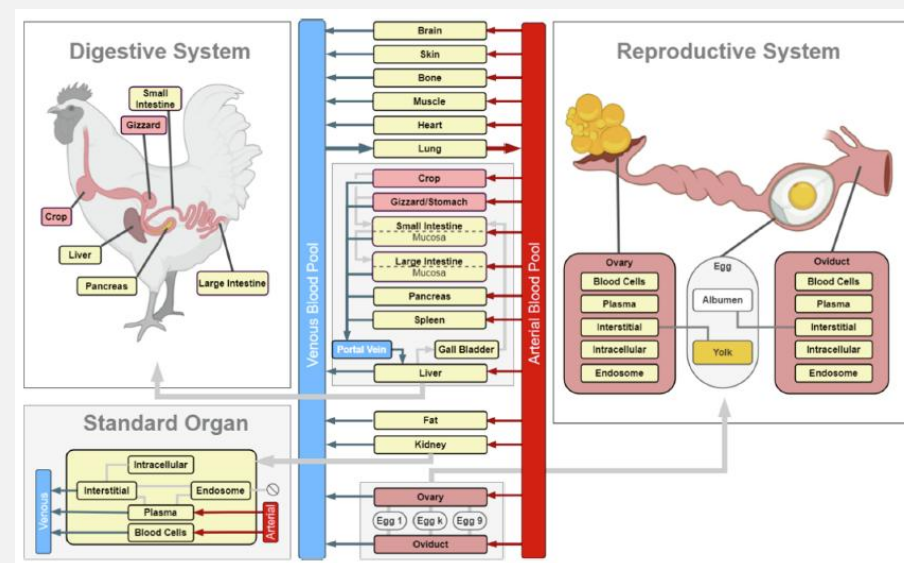
# Aim of this presentation

- § Discuss some potential modelling lines of evidence to address questions around use of fTWA.
- § Inform about principal concepts, data requirements and limitations.

# Modelling approaches - PBK

## § Physiologically-based kinetics (PBK):

- § Predicts internal concentrations in various tissues.
- § Based on intake/exposure over time.
- § Species specific physiological parameters.
- § Chemical specific phys-chem and ADME properties.
- § No effects prediction, exposure only.

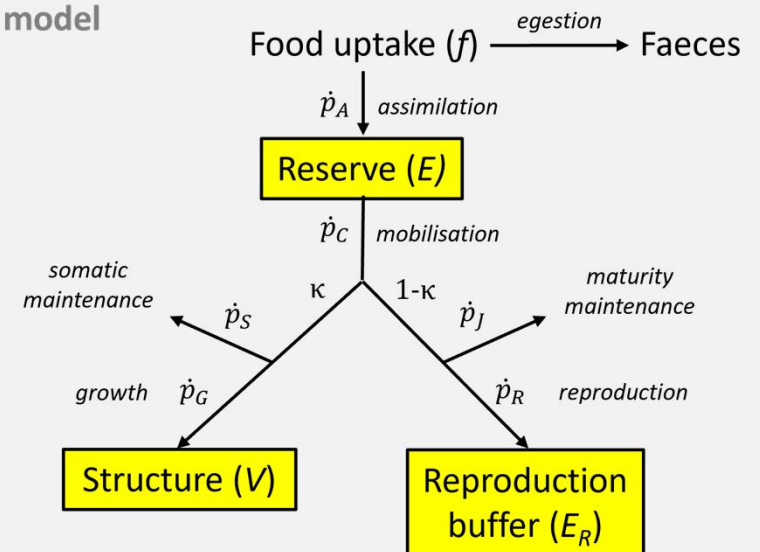


# Modelling approaches - DEB

## § Dynamic Energy Budget (DEB):

- § System of energy flows describing life history of a species
- § Energy allocated to maintenance, growth, maturation or survival
- § No prediction of chemical effects
- § Effects of differences in feeding rate can be modelled

DEB  
model

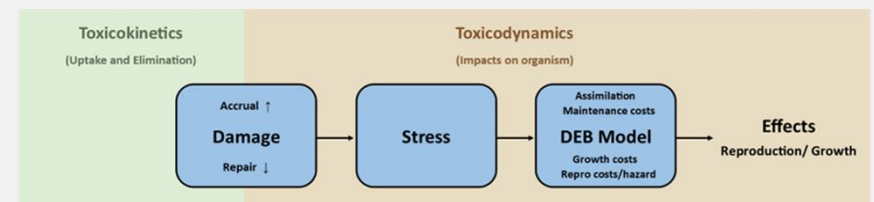
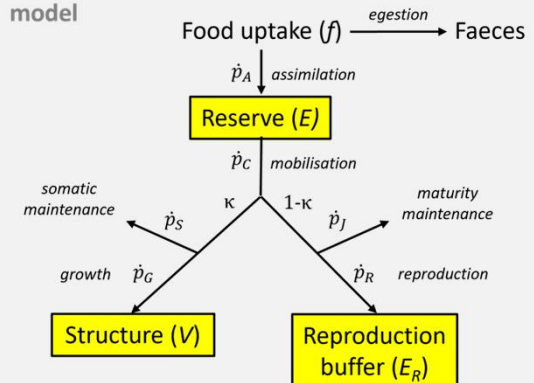


# Modelling approaches - DEB-TKTD

## § Dynamic Energy Budget – Toxicokinetic Toxicodynamic (DEB-TKTD):

- § DEB model with added TK-TD module.
- § TK module (exposure  $\rightarrow$  internal metric) may be PBK model or simpler TK model.
- § TD module simulates disruption of DEB model processes due to internal exposure over time.
- § Models substance related effects on growth, reproduction and maturation.

DEB  
model



# Modelling approaches

What does the model predict?

## § PBK:

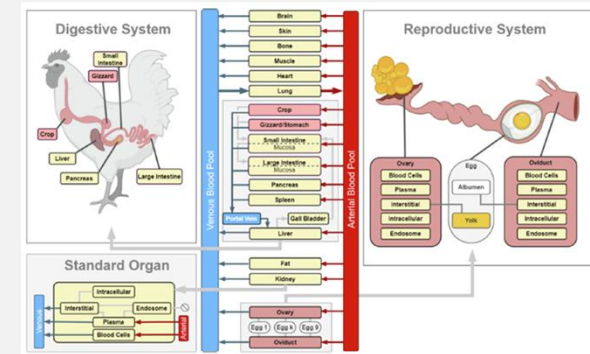
§ Model of internal concentrations based on exposure over time (no effects).

## § DEB:

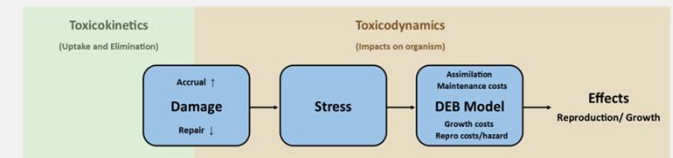
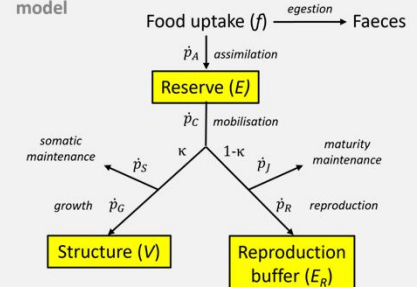
§ Model of growth and reproduction as system of energy flows.

## § DEB-TKTD:

§ Models internal exposure over time (TK) leading to stress on physiological processes (TD) to and sublethal effects (DEB).



DEB model



# Modelling approaches

## Terminology

### § Calibration

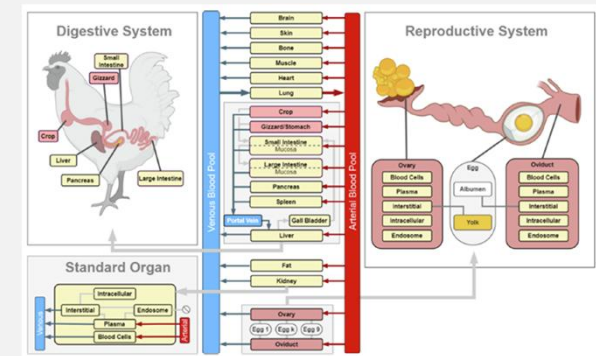
§ Fitting parameter values for best match to observed data.

### § Validation

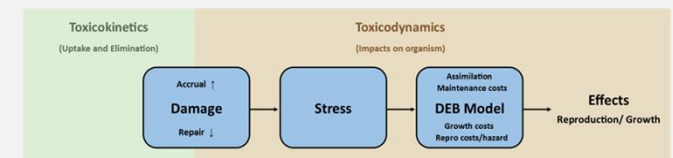
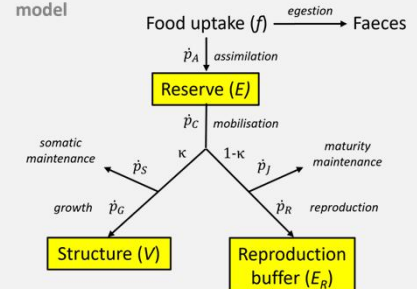
§ Testing model predictions vs independent data (i.e. data not used in model fitting).

### § Prediction

§ Modelling a scenario for which no data are available (e.g. field exposure).



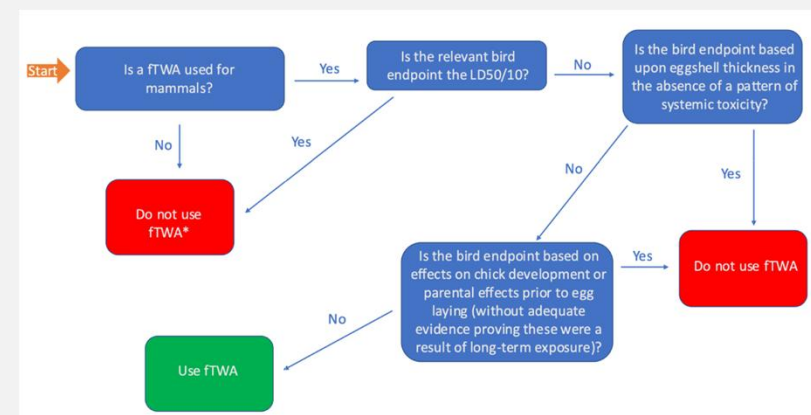
DEB  
model



# Modelling approaches

What question needs to be answered?

- § Are relevant *in ovo* concentrations for developmental effects reached through short-term exposure?
- § Or is long-term exposure needed?





# Evidence for TWA - PBK (Birds)

## § PBK only approach:

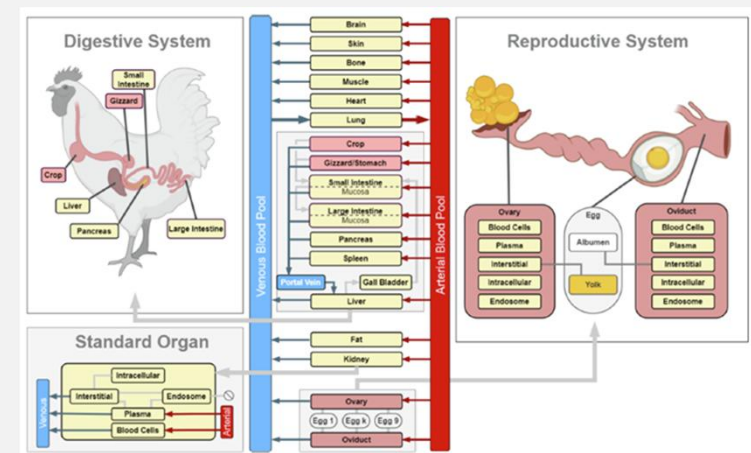
§ PBK modelling can be used to predict *in ovo* concentrations relevant for reproductive effects.

## § Generic avian PBK model (Baier et al., 2022)

§ Models chemical transfer to eggs.

§ Physiological parameters for chicken, mallard duck and bobwhite quail.

§ Requires chemical/ADME parameters, some calibration.



A generic avian physiologically-based kinetic (PBK) model and its application in three bird species

Vanessa Baier<sup>a</sup>, Alicia Paini<sup>a</sup>, Stephan Schaller<sup>a</sup>, Colin G. Scanes<sup>b,c</sup>, Audrey J. Bone<sup>d</sup>, Markus Ebeling<sup>e</sup>, Thomas G. Preuss<sup>e</sup>, Johannes Witt<sup>e</sup>, David Heckmann<sup>e,\*</sup>

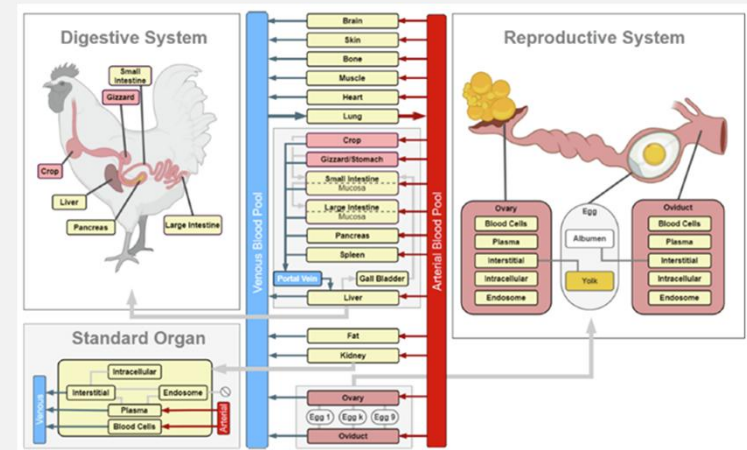
# Evidence for TWA - PBK (Birds)

## § Calibration

- § OECD 503/505 (Metabolism/Residues in Livestock) study provides data for chicken.
- § Not always conducted.

## § Prediction

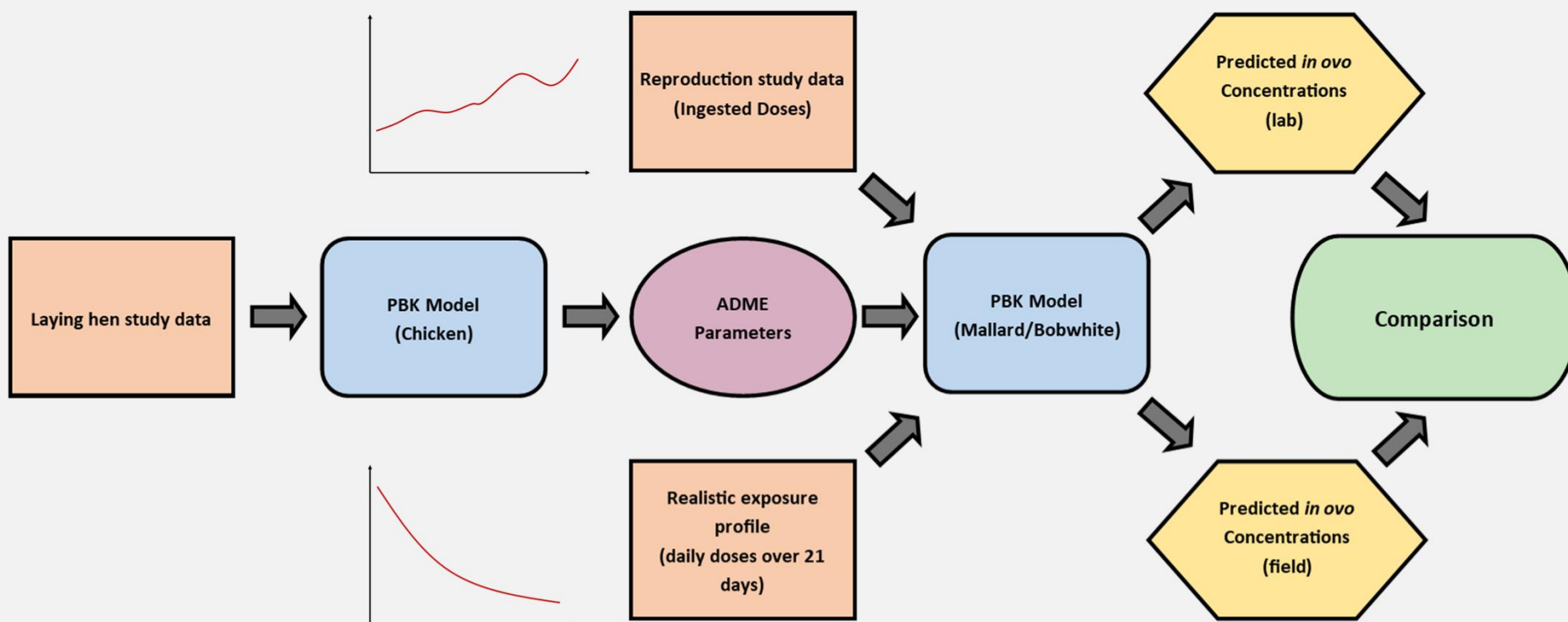
- § Avian reproductive tox study (OECD 206/OCSP 850.2300) on mallard and/or bobwhite quail.
- § At least 10 weeks exposure prior to laying (exposure at all life stages).
- § At least three dose levels.



A generic avian physiologically-based kinetic (PBK) model and its application in three bird species

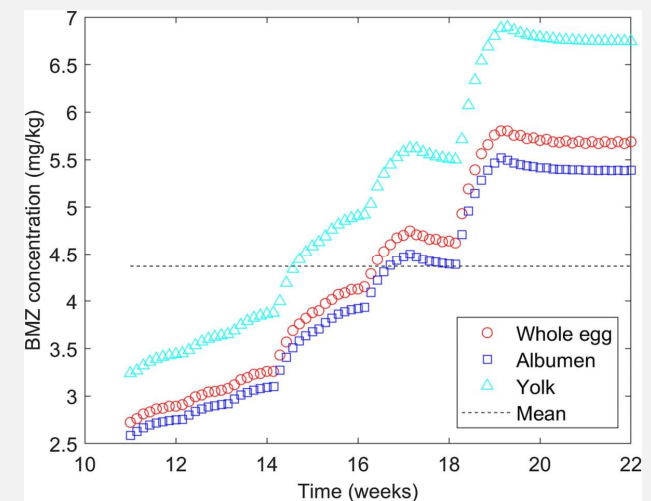
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# Evidence for TWA - PBK (Birds)



# Evidence for TWA - PBK (Birds)

- § Prediction: *in ovo* concentrations in laboratory mallard and/or bobwhite quail reproduction studies.
- § Constant concentration in food.
- § Daily dietary dose fluctuates with feeding rate.
- § Upregulated feeding during reproduction leads to increasing concentrations over egg laying period.
- § *In ovo* concentration >0 for whole laying period, i. e. all embryos exposed.

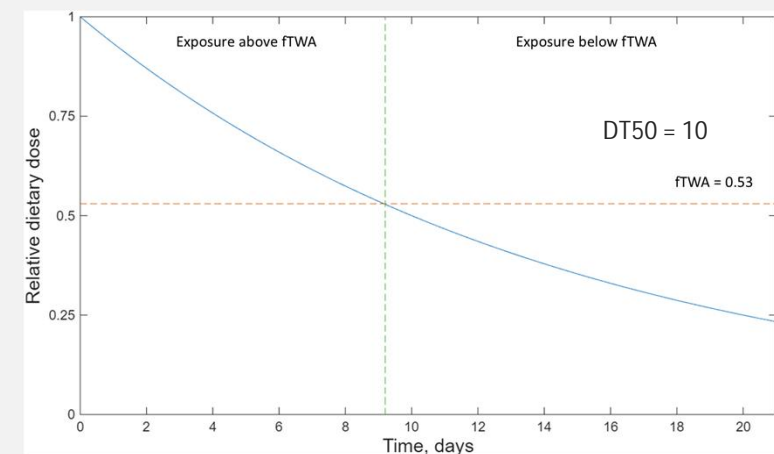


Reproductive toxicity in birds predicted by physiologically-based kinetics and bioenergetics modelling

Thomas Martin<sup>a,\*</sup>, Barbara Bauer<sup>a</sup>, Vanessa Baier<sup>b</sup>, Alicia Paini<sup>b</sup>, Stephan Schaller<sup>b</sup>, Patrick Hubbard<sup>c</sup>, Markus Ebeling<sup>d</sup>, David Heckmann<sup>d</sup>, André Gergs<sup>d</sup>

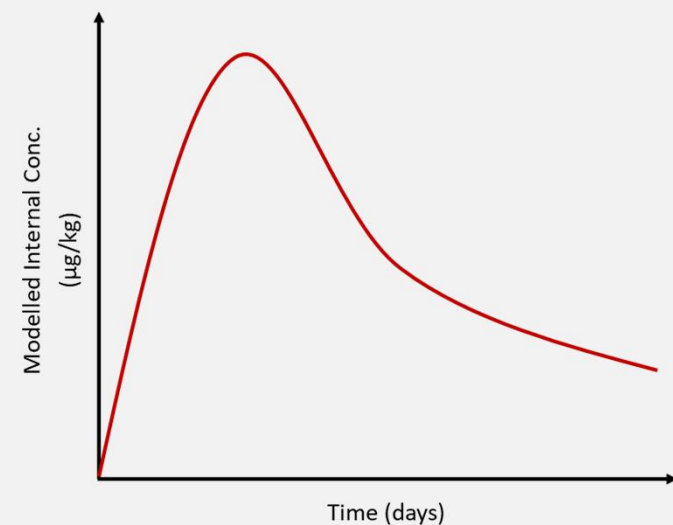
# Evidence for TWA - PBK (Birds)

- § Prediction: realistic dietary exposure profile using a PBK model for mallard/bobwhite quail.
  - § Declining concentration on food items as residues decline after application.
  - § Likely result: initial increase to peak *in ovo* concentration followed by decline.
- § Question: Could peak lead to reproductive effects?



# Evidence for TWA - PBK (Birds)

- § Prediction: realistic dietary exposure profile using a PBK model for mallard/bobwhite quail.
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- § Question: Could peak lead to reproductive effects?



# Evidence for TWA - PBK (Birds)

Comparison of predicted lab and field residues

§ Compare modelled *in ovo* conc. ( $C_{egg}$ )  
between lab and field scenarios.

§ Possible comparisons:

Overlap between  $C_{egg}$  field and  
 $C_{egg}$  NOEL?

$$Max. C_{Egg(field)} < Min. C_{Egg(NOEL)}$$

Compare max  $C_{egg}$  in field and NOEL with  
safety factor

$$\frac{Max. C_{Egg(NOEL)}}{Max. C_{Egg(field)}} \geq X$$

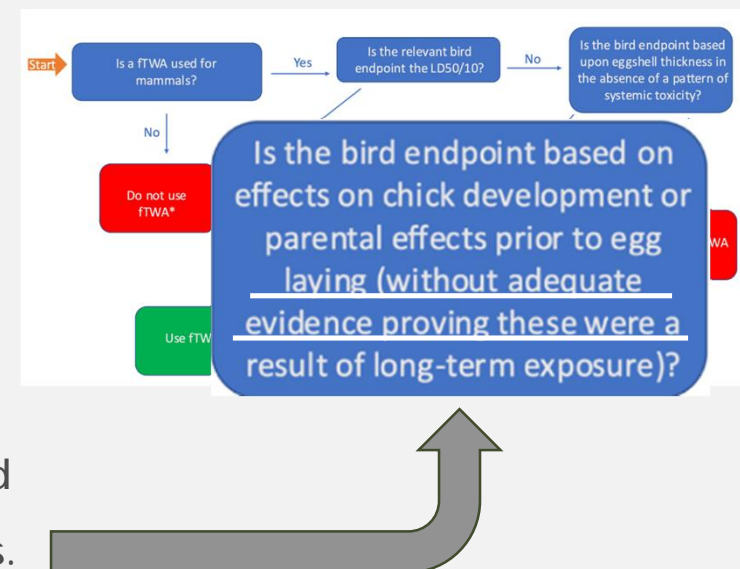
Compare max  $C_{egg}$  in field to min  
 $C_{egg}$  in LOEL with safety factor

$$\frac{Min. C_{Egg(LOEL)}}{Max. C_{Egg(field)}} \geq X$$

# Evidence for TWA - PBK (Birds)

## Summary

- § Calibration and prediction from regulatory data.
  - § Laying hen residue study (if available).
  - § Bird reproductive toxicity study.
- § Applicable for any observed effect.
- § In general, no *in ovo* data available for validation.
- § Meaningful comparison of lab vs field exposure.
  - § Same PBK model for chem/species, only inputs differ.
- § Can provide evidence long-term exposure required to reach *in ovo* concentrations relevant for effects.



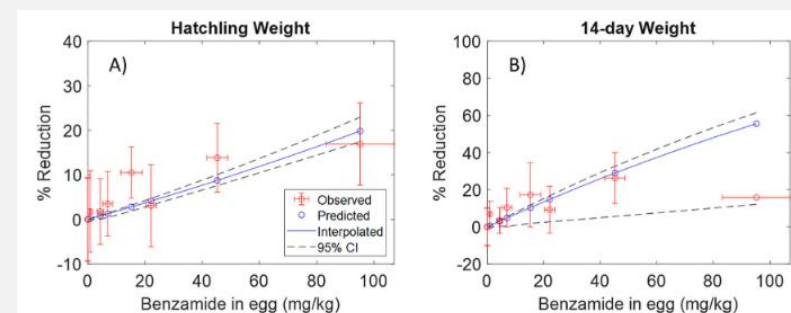
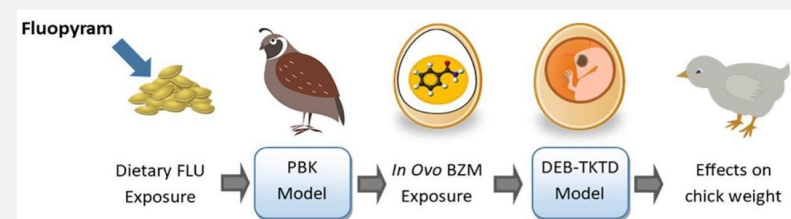


# Evidence for TWA - PBK + DEB-TKTD

Case study (real substance)

## § Martin et al. (2024)

- § Calibrated DEB-TKTD model to embryo growth in egg injection study.
- § *In ovo* concentrations in OECD 206 modelled with PBK.
- § Effects on hatchling and 14-day weights predicted accurately.

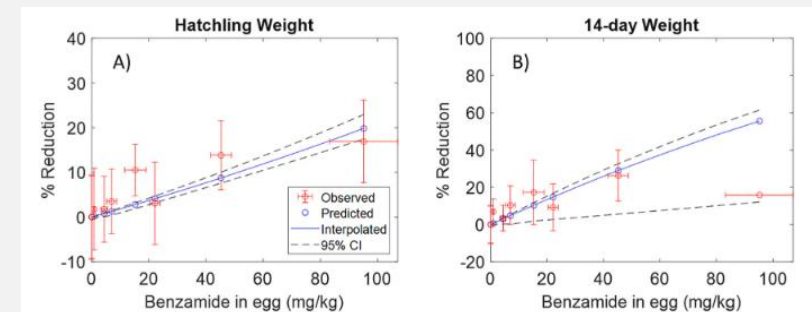
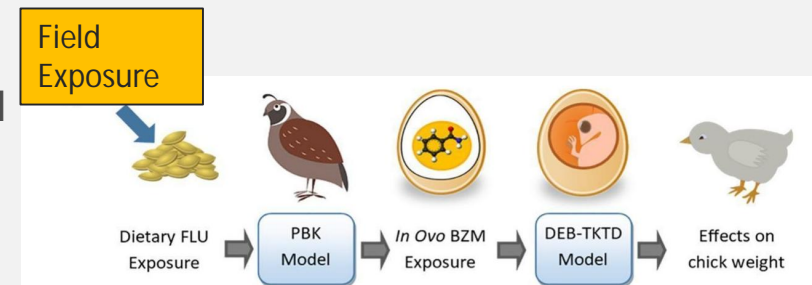


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# Evidence for TWA – PBK + DEB-TKTD

- § Predict *in ovo* concentrations from realistic field exposure.
- § Compare modelled *in ovo* concentrations to *in ovo* dose response curves.
  - § Evidence for/against TWA.
  - § Short-term exposure sufficient for effects?
- § Predict effects over 21-day window
  - § *In silico* pulse experiment.
  - § Multiply exposure for X% effect.

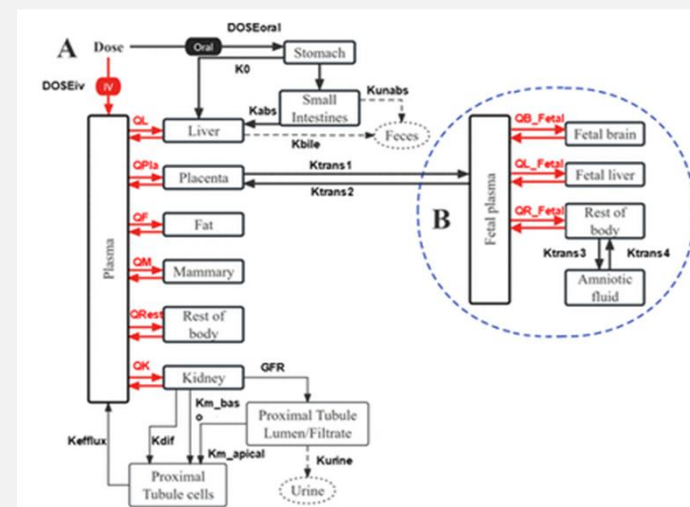


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# PBK modelling - Mammals

- § Mammal offspring exposure more complex and dynamic.
  - § Initially via placenta, then via milk.
- § Standard kinetics study (OECD 417) does not include pregnant females.
- § PBK modelling is more complex, as is comparison between lab and field predictions.



**Development of a Physiologically Based Pharmacokinetic (PBPK) Model for F-53B in Pregnant Mice and Its Extrapolation to Humans**

Jing Zhang, Shen-Pan Li, Qing-Qing Li, Yun-Ting Zhang, Guang-Hui Dong, Alexa Canchola, Xiaowen Zeng,\* and Wei-Chun Chou\*

# Evidence for TWA – DEB

What question needs to be answered?

- § Are observed effects on reproduction primary?
- § To what extent can effects be explained by differences in parental food intake?

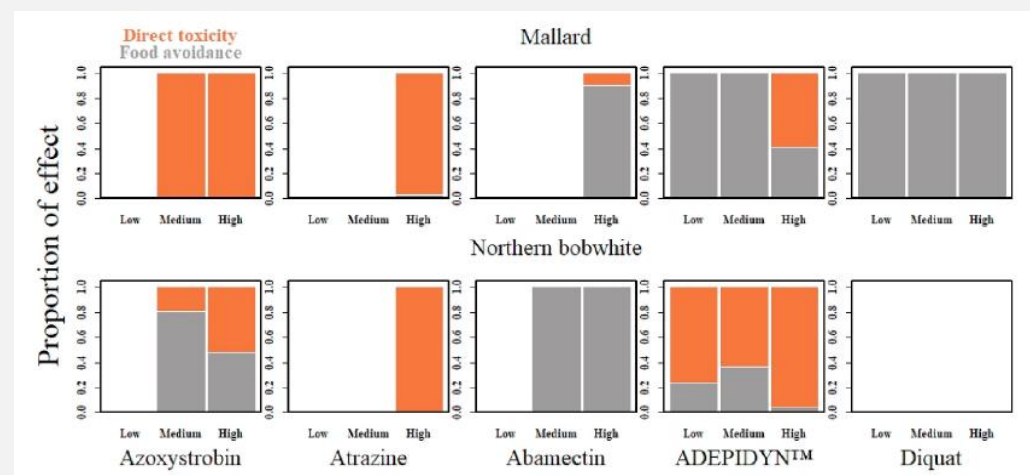
	Mammals	Birds
<b>Effects for which the TWA is appropriate</b>	Body weight and body weight change Food intake Liver and kidney effects Other organ-level effects (See Section 5.2.6.5)	Effects other than those specified in the following rows of this column
<b>Effects for which case-by-case expert judgement should be employed</b>	Effects on reproduction/development with some (parental) body weight or slight toxic effects  Some tumours (see explanatory text above)	Effects on chick development which may be primary effects (i.e. unless a clear pattern of maternal toxicity is proven)  Effects on parental birds as represented by body weight changes in females prior to egg laying (see explanatory text above)
<b>Effects for which the TWA should not be used</b>	Primary reproductive effects (consult with mammalian toxicology and see Glossary and abbreviations); i.e. effects on fertility and fecundity not as a result of systemic toxicity  Primary developmental effects (already judged sufficient for endpoint setting see Section 5.2.6.5); e.g. developmental effects in the absence of parental or systemic toxicity	Effects on eggshell thickness not correlated maternal bw/bw change/ systemic toxicity  When the LD <sub>50</sub> /10 is lower than the endpoints from the avian reproduction study

# Evidence for TWA - DEB (Birds)

Case study (real substances)

## § Trijau et al. (2023)

- § Reduced egg laying in OECD 206 studies predictable from observed feeding reduction for Abamectin and Diquat
- § TKTD model not needed (DEB only predictions)
- § Evidence that reduced egg laying is a secondary effect?



Development of a mechanistic model for analyzing avian reproduction data for pesticide risk assessment<sup>☆</sup>

Marie Trijau<sup>a</sup>, Benoit Goussen<sup>a,\*</sup>, Richard Brain<sup>b</sup>, Jonathan Maul<sup>b</sup>, Nika Galic<sup>b</sup>

# Evidence for TWA - DEB (Mammals)

- § Same approach possible for mammals.
- § Similar published example (right) considered growth effects only.
- § DEB model for Wistar rat including reproduction is publicly available.
- § Maternal energy supply throughout gestation and lactation is critical.



ARTICLE | November 1, 2019

## Toxicokinetic–Toxicodynamic Modeling of the Effects of Pesticides on Growth of *Rattus norvegicus*

Thomas Martin\*, Helen Thompson, Pernille Thorbek, and Roman Ashauer

# Evidence for TWA – DEB (Birds & Mammals)

- § DEB can predict growth and reproduction based on observed feeding.
- § Can provide evidence that effects on reproduction or development are secondary, without clear parental toxicity.
- § May be most relevant for post-partum effects (e.g. delayed maturation, reduced growth) in mammals.

# Modelling approaches: DEB-TKTD

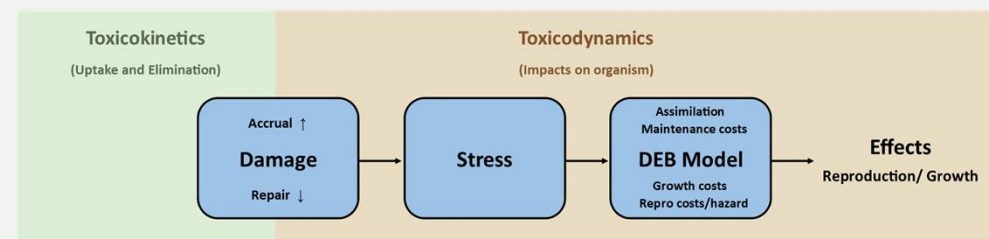
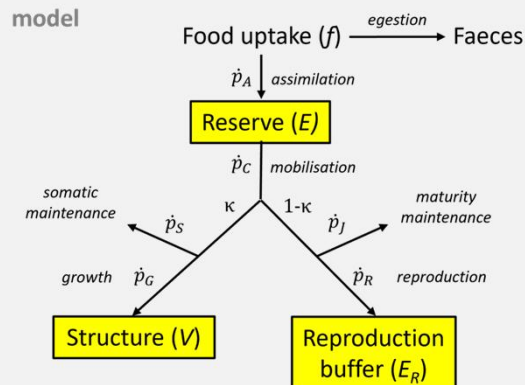
## § What DEB-TKTD modelling can do:

- § Model effects of novel exposure scenarios, based on available data (i.e. alternative to TER approach).
- § Predict whether effects occur following short-term exposure.

## § What DEB-TKTD modelling cannot do:

- § Prove a negative.
- § Show that short-term exposure CANNOT cause effects.

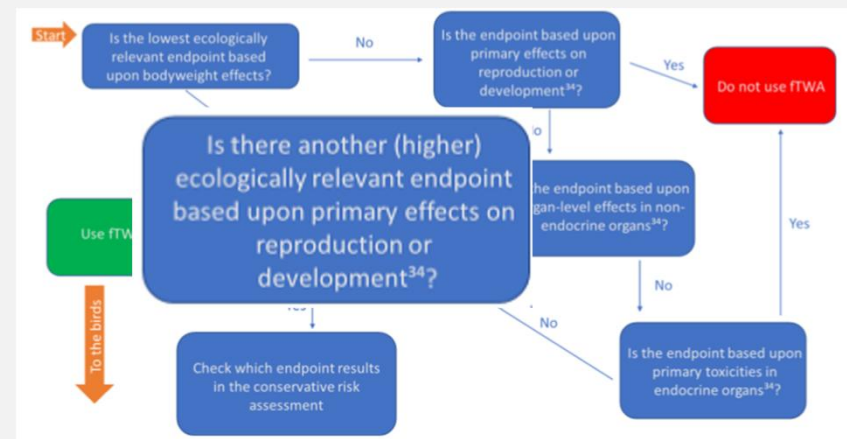
DEB  
model





# Evidence for TWA - Ecological relevance

- § Could short term exposure result in ecologically relevant effects?
- § Depends on presence/absence of individuals at vulnerable life stage



# Evidence for TWA - Ecological relevance

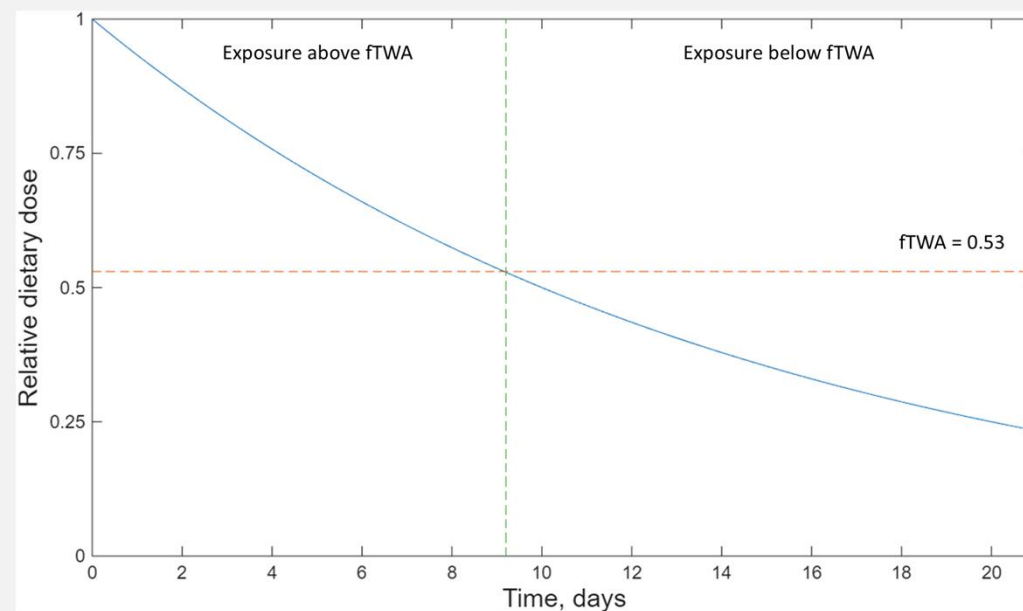
§ Assuming  $DT_{50} = 10$  and a 21-day relevant exposure window.

§ 9.2 days above fTWA.

§ 11.8 days below fTWA.

§ If  $DT_{50} < 10$

§ Smaller proportion of 21-day window above fTWA.



# Evidence for TWA - Ecological relevance

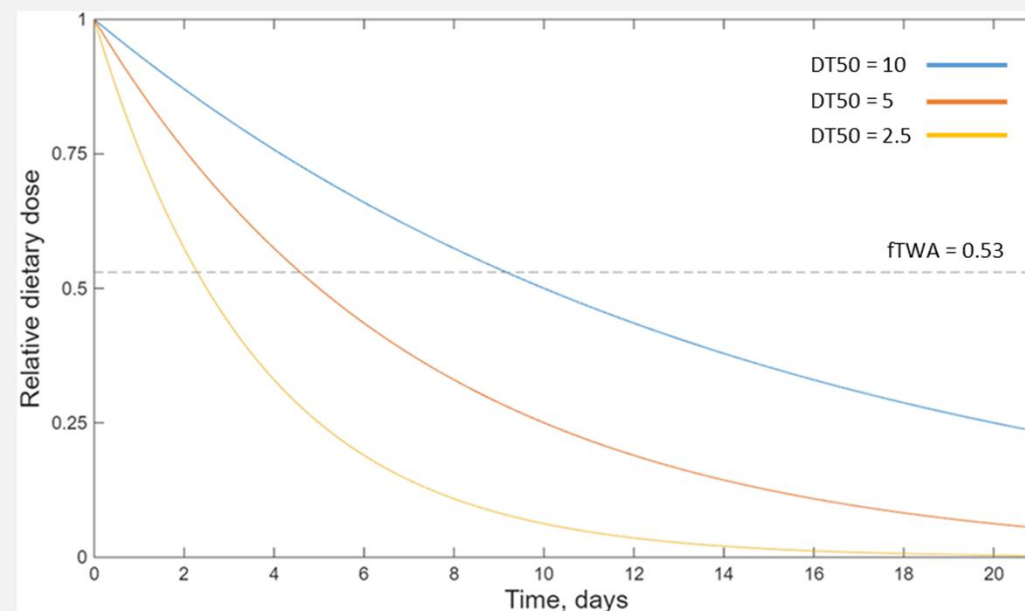
§ Assuming  $DT50 = 10$  and a 21-day relevant exposure window.

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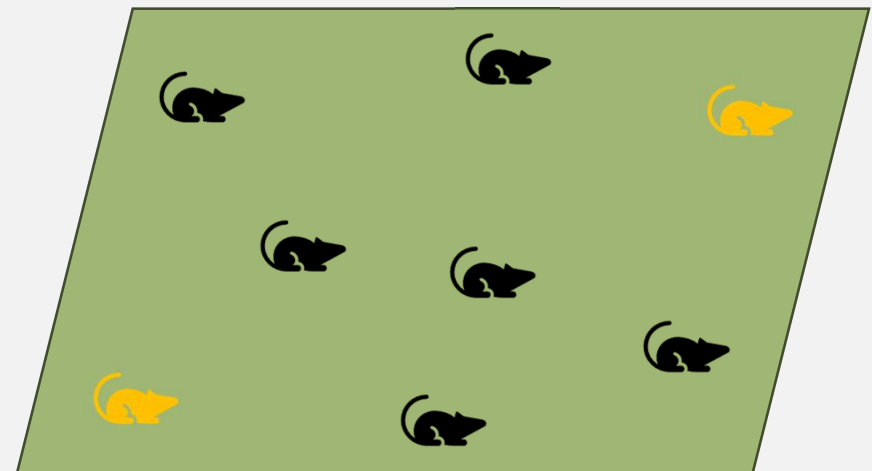


# Evidence for TWA - Ecological relevance

## Population modeling

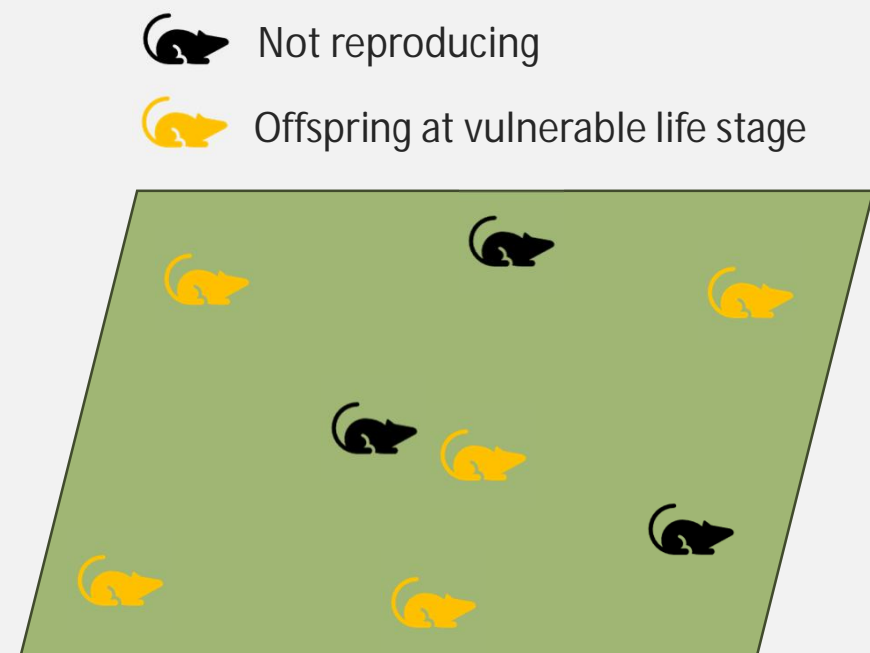
- § Model short peak exposure in population model, assuming toxicity from short-term exposure.
- § What proportion of population have offspring at vulnerable life-stage?
- § What is effect size at population level?

-  Not reproducing
-  Offspring at vulnerable life stage





# Evidence for TWA - Ecological relevance

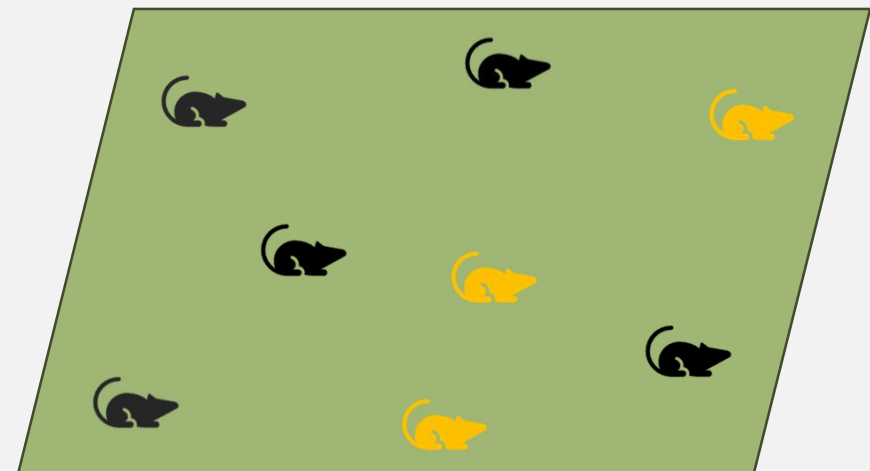
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# Evidence for TWA - Ecological relevance

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- § What proportion of population have offspring at vulnerable life-stage?
- § What is effect size at population level?

-  Not reproducing
-  Offspring at vulnerable life stage



## Summary

- § PBK modelling can provide evidence that relevant internal concentrations are not reached from short-term exposure.
- § DEB modelling alone may show effects are predictable due to reduced feeding – secondary effect.
- § TKTD modelling simulates toxic effects based on available (long-term) data.
  - § If data do not prove there is no short-term toxicity, neither can models.
- § Population modelling can estimate population-level effects resulting from short-term exposure.

# Summary

- § Modelling is not standard, but “higher tier” approach for fTWA argumentation
- § No approach can be universally applied
  - § Available data and questions to address vary from compound to compound
  - § Case specific approaches often required
  - § Promising approaches - but not easily applicable on short-notice
- § Acceptance by authorities uncertain



# Thank You

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