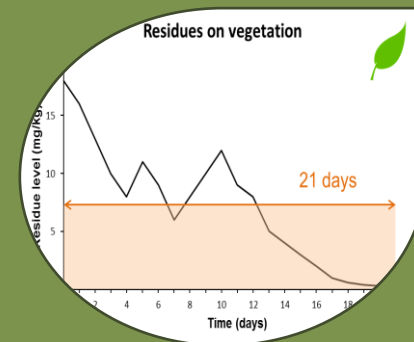


3rd Virtual Workshop on the revised EFSA Birds and Mammals Guidance Document

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



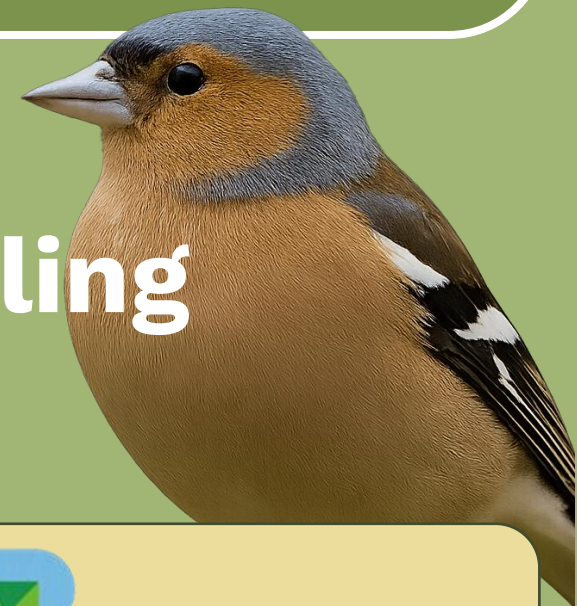
April 28, 2026



10 am – 3 pm CET

Steph Plautz - CLE

Topic 3a: Non-modelling lines of evidence



RIFCON



CropLife
EUROPE

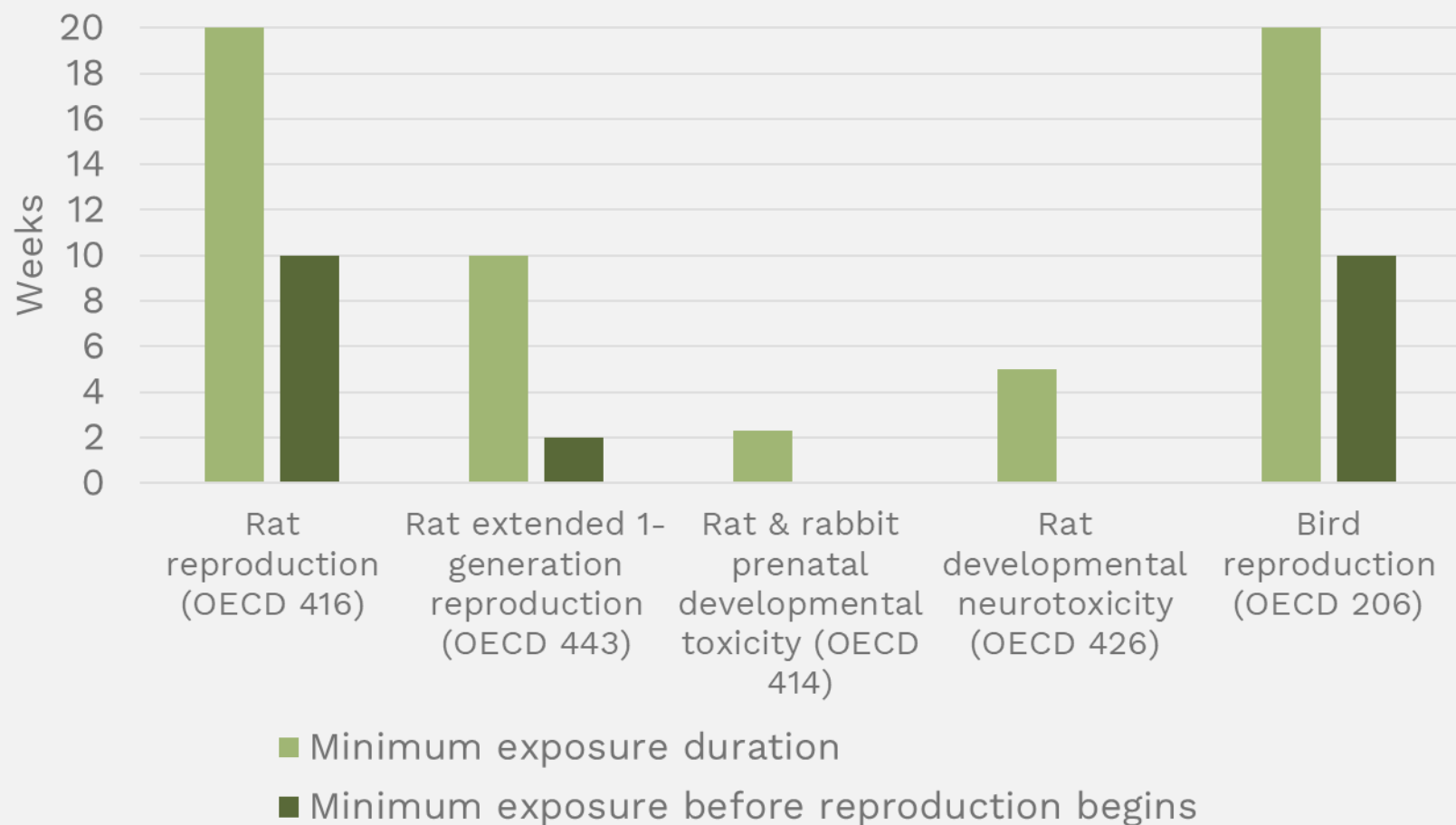
Aim of this presentation



- Draw attention to why additional lines of evidence may be useful in fTWA evaluations
- Discuss some potential non-modelling lines of evidence
 - See the presentation on modelling lines of evidence

Why is additional data needed?

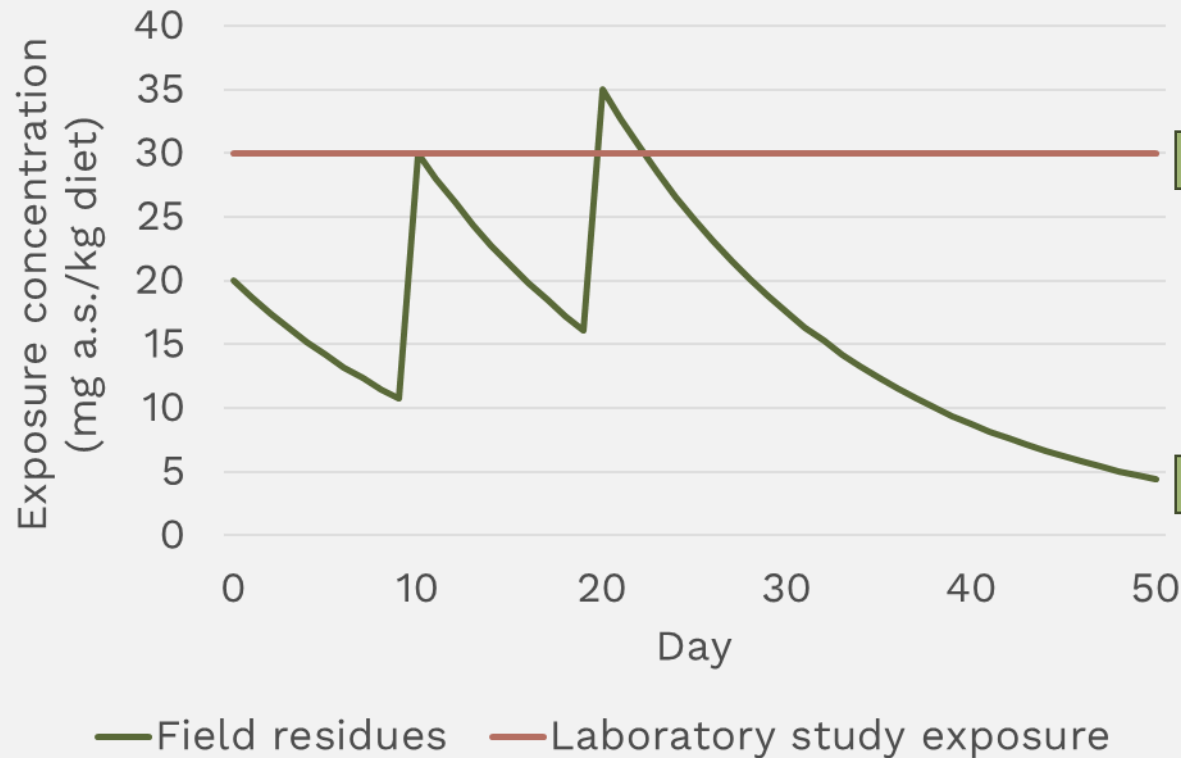
Study exposure timing and duration



Based on current study designs, in most cases it is not possible to know whether effects on reproduction or development are due to long-term exposure (fTWA appropriate) or short-term exposure (fTWA not appropriate)

Why is additional data needed?

Exposure in lab studies versus field



Laboratory studies:

Relatively continuous exposure for an extended time (minor fluctuations due to different reproductive phases)

Field residues:

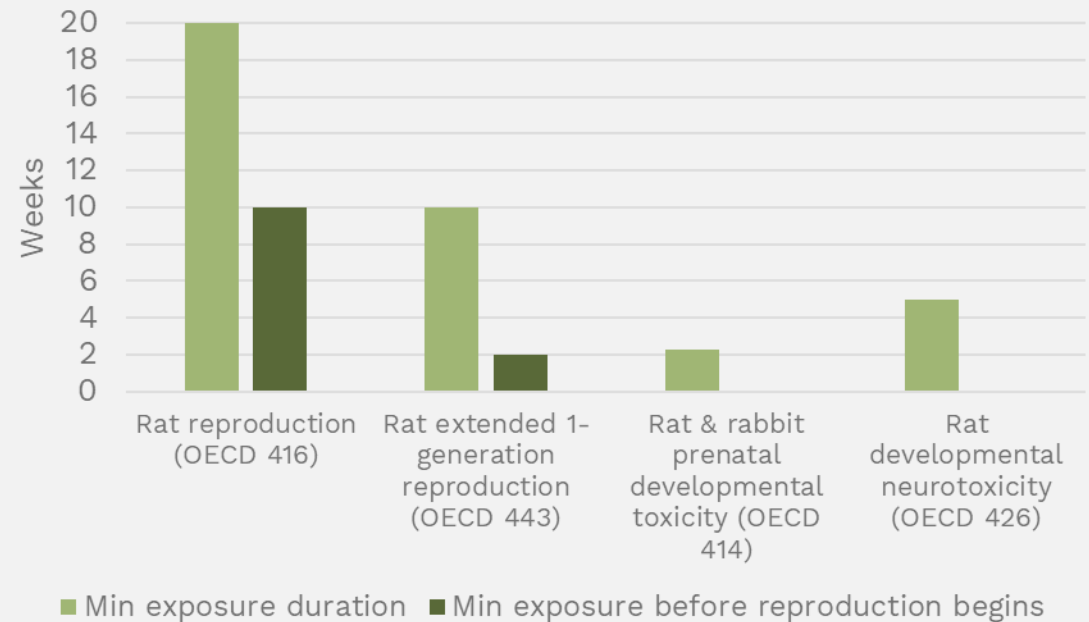
Decline over time following application(s)

Modelling can help to determine whether the effects reported in lab studies would also be seen in the field, when NOAEL/BMD₁₀ exceedances may be short. See the presentation on modelling lines of evidence.

Mammals: Exposure duration varies with study type

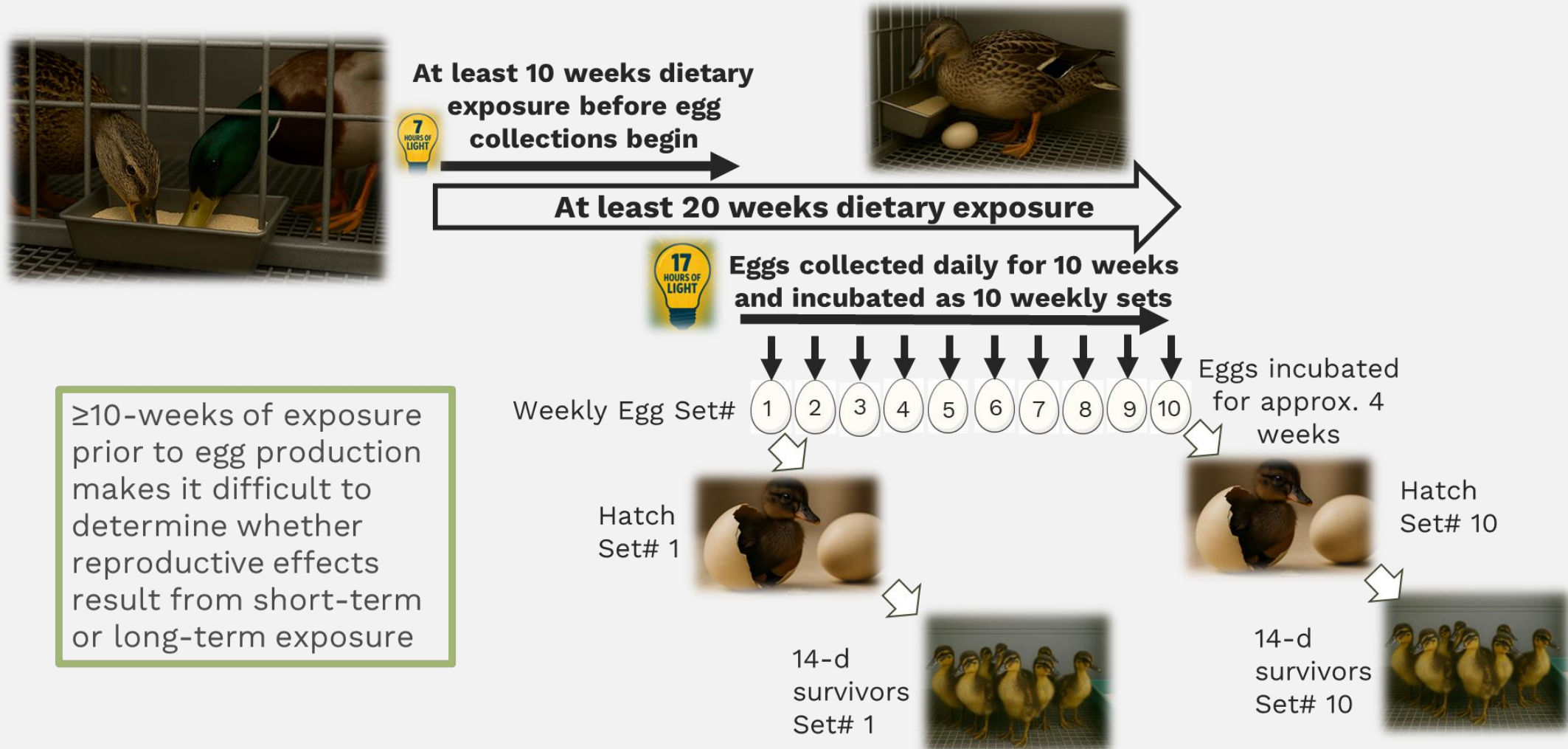
Multiple study types that include reproduction and/or developmental endpoints may be available for mammals:

- Rat multi-generation reproduction
- Rat extended one-generation reproduction
- Rat developmental neurotoxicity
- Rat and rabbit developmental toxicity



These studies have varying exposure durations before and during reproduction → **other studies can help to contextualize effects seen in individual studies**

Birds: Standard reproduction study design

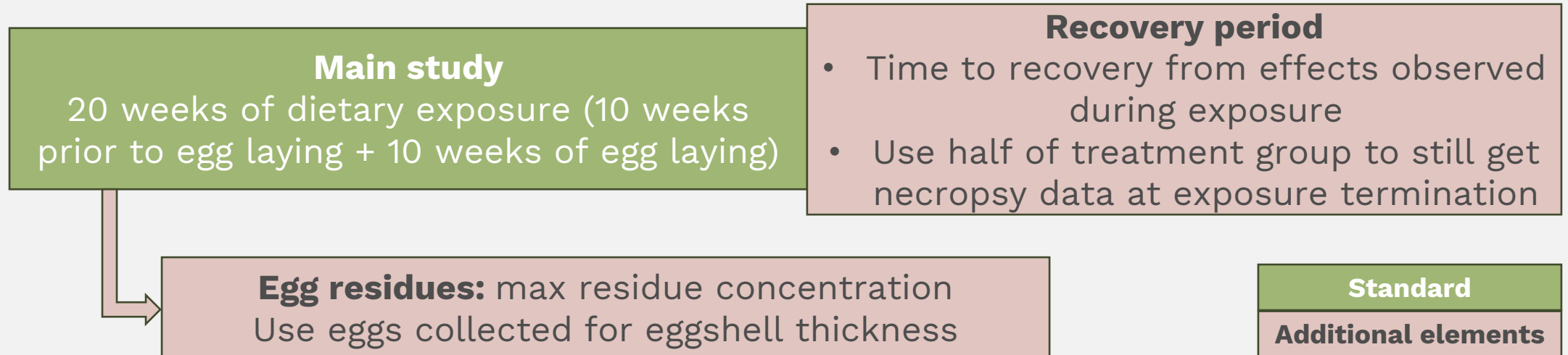


Birds: Modified reproduction study design (OECD 206/OCSP 850.2300)



- Standard study design makes it difficult to determine whether effects result from short-term or long-term exposure → Can the study design be modified?
- **Challenges**
 - Existing studies should not be repeated → study design modifications are only useful for new active substances or studies being repeated due to deficiencies
 - Modifications should not alter global study acceptability
 - Modified study design would not directly answer the question of whether fTWA use is appropriate → only provides information for additional lines of evidence

Birds: Modified reproduction study design (OECD 206/OCSP 850.2300)



Data	Use for fTWA evaluations
Egg residue max concentration	Can be used to set doses in an egg injection study
Recovery time	Can be used in modelling

Birds: Other experimental data



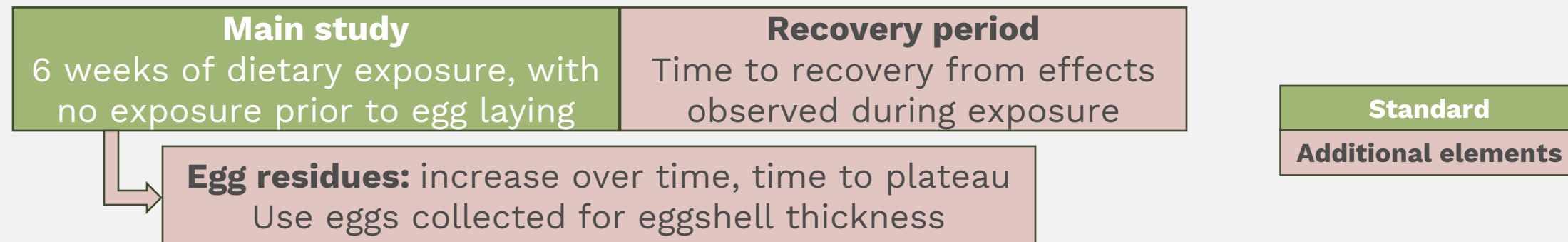
Reproduction screen based on OECD 206/OCSPP 850.2300

- Reproduction screens are sometimes conducted as range finders to help set dietary concentrations for bird reproduction studies
- Not available for all active substances or for all bird species tested for an active substance
- **Challenges**
 - Existing studies should not be repeated → study design modifications are only useful for new active substances
 - Conducted with fewer replicates than a definitive study → low statistical power
 - Often conducted non-GLP → data acceptance?
- **Opportunity:** may already answer the question for some parameters of whether effects are due to short-term or long-term exposure

Birds: Other experimental data



Reproduction screen based on OECD 206/OCSP 850.2300



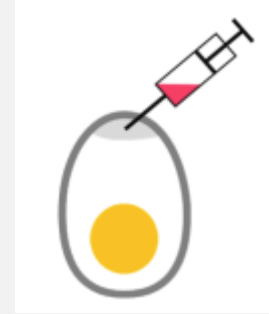
Data	Use for fTWA evaluations
Increase in reproductive effects over time (if any effects)	Exposure time for effects to plateau or reach population relevance threshold
Egg residues over time	Time to reach an egg residue concentration that may have effects on developing embryos
Egg residue plateau concentration	Can be used to set doses in an egg injection study
Recovery time	Can be used in modelling

Birds: Other experimental data

Egg injection studies

What is an egg injection study?

- Test substance is injected into the air cell of fertilized eggs
- Injection hole in the shell is sealed & egg is incubated normally



Use in fTWA evaluations

- Demonstrate whether there is a link between effects reported in a bird reproduction study and egg residues (cannot address all endpoints from bird reproduction studies)
- If yes, how long does it take for this egg residue concentration to be reached?

How can egg injection study doses be set?

- Egg injection range finder
- Egg residue measurements from bird reproduction screens and/or definitive studies (typically not available in existing studies)
- PBK modelling (see the presentation on modelling lines of evidence)

Birds: Other experimental data



Egg injection studies

Opportunity: If an egg injection study is terminated before the embryos are considered vertebrates, this may be considered a non-vertebrate study

Challenges

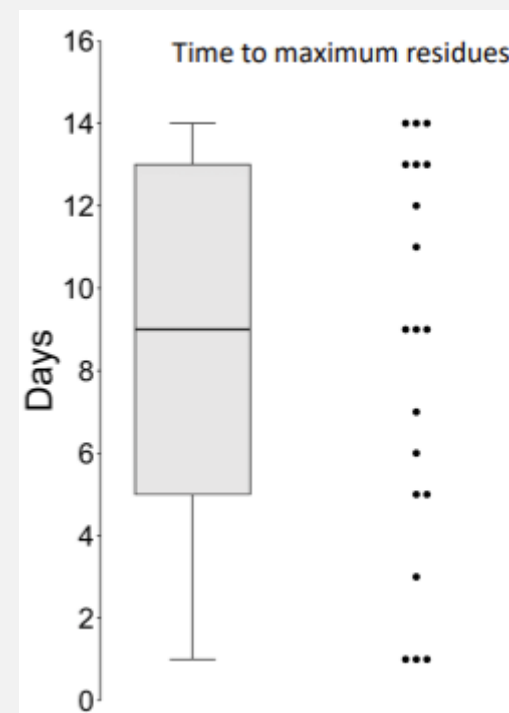
- Not all parameters in a bird reproduction study can be included in an egg injection study, particularly when it is terminated before embryos are considered vertebrates
 - Time point at which developing embryos are considered vertebrates varies by country and over time
- Effects on developing embryos in the reproduction study may result from metabolite exposure, not only exposure to active substance
- Data on egg residues (active substance and/or metabolites) over time are typically not available unless a laying hen metabolism and residue studies are available
- Regulatory acceptance is uncertain for egg injection studies (no standard approach) and for PBK modelling to potentially set test doses & aid in risk assessment

Birds: Other experimental data

Egg injection studies

Laying hen metabolism and residue studies

- Conditionally required for active substances → no additional vertebrate testing
- Eggs collected daily & analyzed for active substance (and possibly metabolite) residues
- Time course of residue deposition varies by compound: median 9 days to plateau for 19 test substances in a dataset compiled by CLE



Left: boxplot of the time to maximum egg residue

Right: asterisks illustrating the data distribution

Ebeling et al. (2023)
Laying hen studies and time-to-effect in reproductive risk assessments for birds. Poster presentation at SETAC Dublin

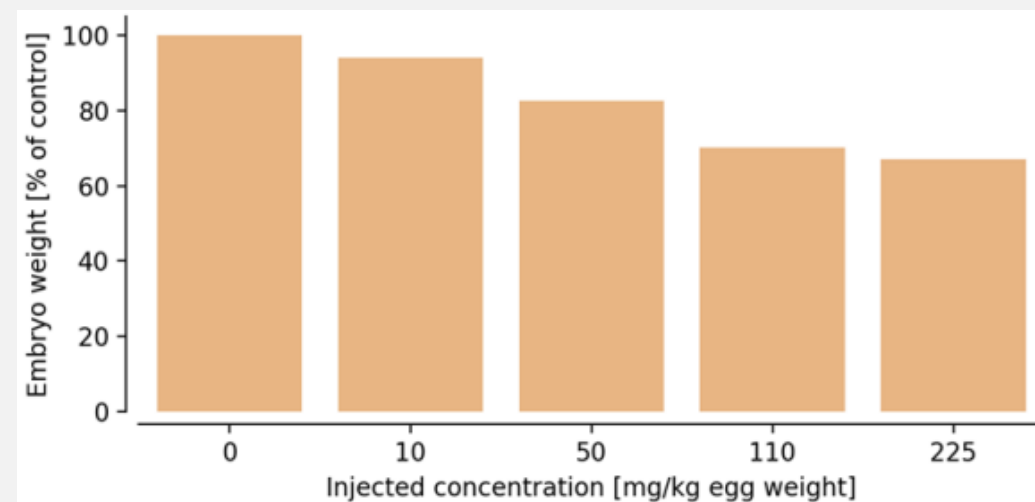
Useful information for fTWA evaluations

- Identification of egg residues (active substance and/or metabolite)
- Time course of egg residue concentrations, including time to plateau

Birds: Other experimental data

Egg injection studies (real active substance)

1. Bobwhite quail reproduction study showed reduced hatchling weight compared to controls
2. Laying hen metabolism study used to identify the residues (active substance + metabolites in this case) being deposited in eggs
3. Similar ↓ embryo weight in egg injection study compared to bobwhite reproduction study
4. DEB-PBK model developed using time course of egg residue increase from laying hen study and egg injection toxicity data. Model was validated by comparing its predictions to the chick weight results of the reproduction study → they matched.



Ebeling et al. (2022) Laying hen studies and time-to-effect in reproductive risk assessments for birds. Poster presentation SETAC Copenhagen.

Conclusion: All together, this data supports fTWA use in the bird reproductive risk assessment because of the time to reach egg residues that resulted in toxicity. Approaches can be tailored to the compound and effects seen in the bird reproduction study.

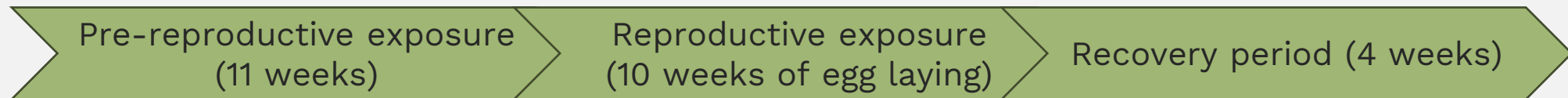
Birds: Eggshell quality

- ↓ eggshell thickness driving the bird reproduction NOAEL/BMD₁₀ means fTWA cannot be used → implies that effects on eggshell thickness are due to short-term exposure
- Calcium used for eggshell formation is obtained via uptake in the intestines and/or resorption from bones
- When birds lay eggs over an extended time period (≥10 weeks in bird repro studies), supplemental dietary calcium is required
- Eggshell deposition occurs in <1 day, but toxicants may interfere with eggshell formation via multiple mechanisms, which cannot be determined from a standard bird reproduction study

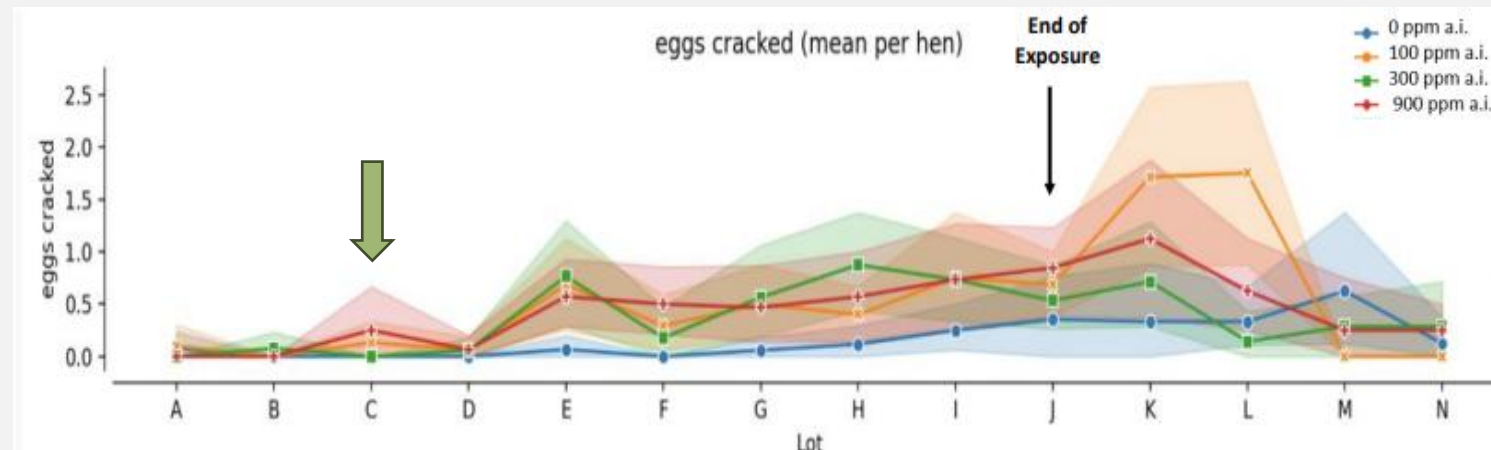
Proposal: Evaluate effects on eggshell quality on a case-by-case basis

Birds: Eggshell quality evaluation (real active substance)

1. Non-standard study design



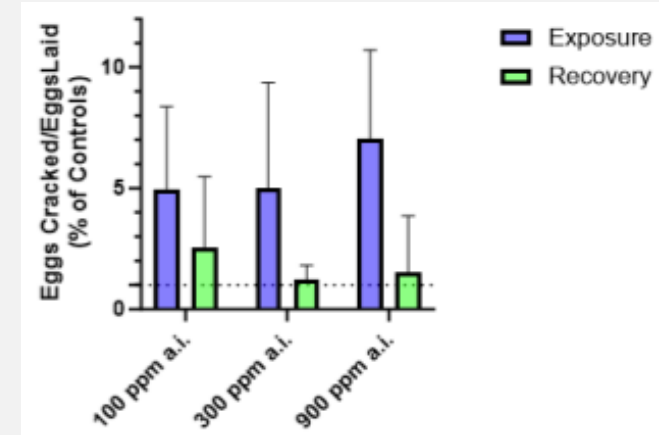
2. Dietary exposure period: ↑ cracked eggs/hen/week starting ~2-3 weeks after the start of egg laying (this equals 13-14 weeks after the start of dietary exposure), with increasing effects over time. Eggshell thickness was unaffected.



Endpoint	0 ppm a.i.	100 ppm a.i.	300 ppm a.i.	900 ppm a.i.
Eggs cracked of eggs laid	0.025	0.118*	0.092*	0.143*
Eggshell thickness (mm)	0.228	0.220	0.219	0.213

Birds: Eggshell quality evaluation (real active substance)

3. Recovery period: recovery of eggshell cracking within 2-3 weeks after dietary exposure ended



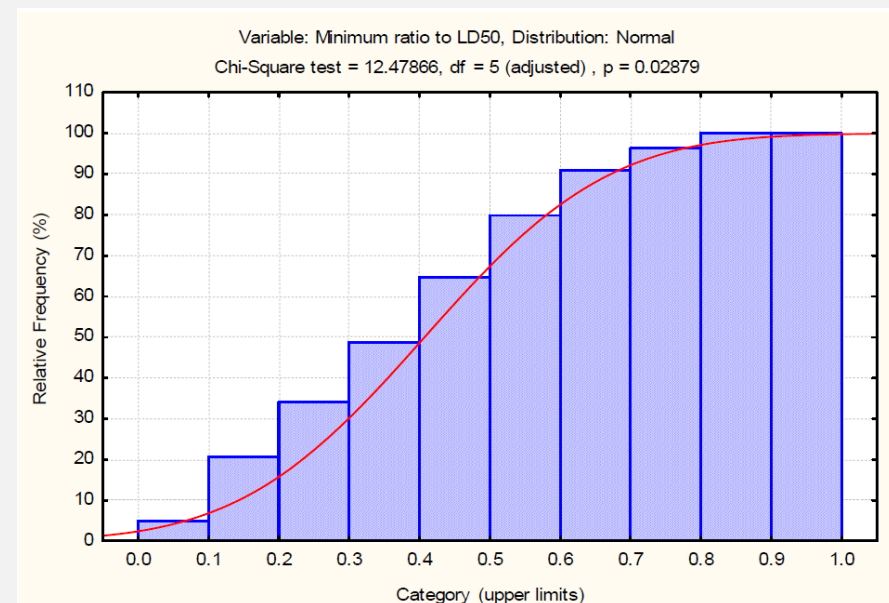
Conclusions:

1. Timing of effect onset for cracked eggs/egg laid (after 13-14 weeks of exposure) suggests that the test substance stressed the calcium balance of the birds following extended exposure
2. Exposure window for effects on eggshell quality may be much longer than 1 day

Birds: LD₅₀/10

- If LD₅₀/10 is the relevant endpoint for reproductive risk assessment → no fTWA
- Derived from a comparison of sublethal effect LOELs to the LD₅₀ in acute oral studies (Callahan & Mineau 2000 in EFSA Journal (2008) 734, 2-181)
 - Dataset: 116 pesticides (171 studies) registered in Canada and US 1962-1996, including many organophosphates and carbamates
 - Only 18 of 116 pesticides still registered in EU
 - Currently registered pesticides generally show lower toxicity to birds
- Substance-specific acute studies often provide information to assess potential sublethal dose levels

Proposal: Case-by-case evaluation of active substance bird data



“Based on this analysis, a cutoff value of 0.1 for the ratio of the first sign of toxicity to the LD₅₀ would have been ‘protective’ in approximately 95% of the studies. A value of 0.1 of the LD₅₀ is therefore proposed as the value to use as an estimate of the dose which may cause impairment and possibly endanger a reproductive effort in the field.”
(Callahan and Mineau 2000)

Birds: LD₅₀/10 evaluation

(real active substance)

Study type	Test species	LD ₅₀ (mg a.s./kg bw)	Sublethal effects (mg a.s./kg bw (/d))	
			NOEL	LOEL
Acute oral	Japanese quail	1050	175	290
	Canary	>1510	305	520
Reproduction	Bobwhite quail	n/a	130	>130

- LD₅₀/10 of 105 mg a.s./kg bw < reproduction NOAEL of 130 mg a.s./kg bw/d
- However, lowest sublethal effects NOEL of 175 mg a.s./kg bw from the acute oral studies

Conclusions:

1. Reproduction NOAEL is protective of sublethal effects in the acute oral studies, so the appropriate reproductive risk assessment endpoint is NOAEL = 130 mg a.s./kg bw/d
2. fTWA can be used based on this endpoint (no effects were reported)

Summary

- Time to effect for developmental and reproductive parameters typically cannot be determined from current bird & mammal studies
- Potential non-modelling sources of additional information:

	Mammals	Birds
Existing data	1. Multiple study types that include reproduction and/or developmental endpoints and varying exposure durations	1. Reproduction screen (not available for all active substances/species) 2. Laying hen studies (conditionally required) 3. Evaluation of active substance-specific data when $LD_{50}/10 < \text{repro NOAEL}$
Potential new data	Ecotox cannot change standard toxicology study designs	1. Reproduction screen using a modified design (new actives only) 2. Reproduction study using a modified design (new actives and studies repeated because of deficiencies only) 3. Egg injection studies

Thank You

www.rifcon.de | LinkedIn: Rifcon