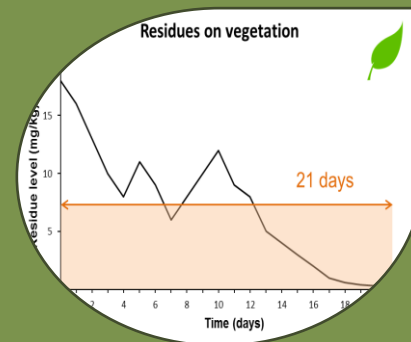


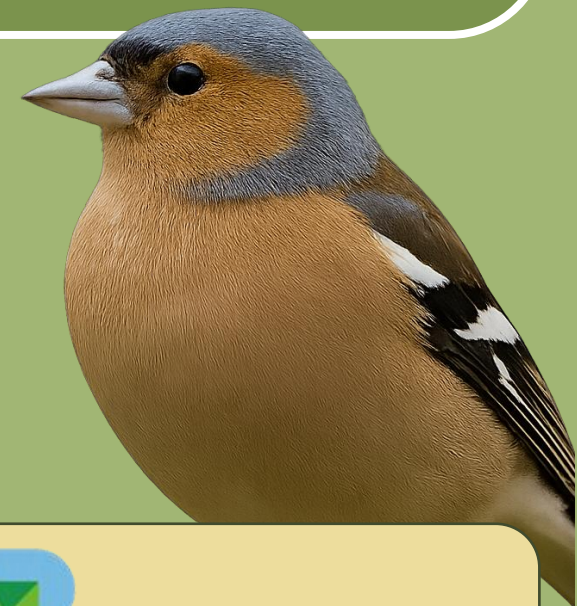
3rd Virtual Workshop on the revised EFSA Birds and Mammals Guidance Document

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



Michail Gioutlakis - RIFCON

Topic 2: Ambiguities



RIFCON



CropLife
EUROPE

Aim of this presentation

Draw attention to ambiguities in the birds and mammals guidance document (EFSA 2023) regarding fTWA applicability in reproductive risk assessments:

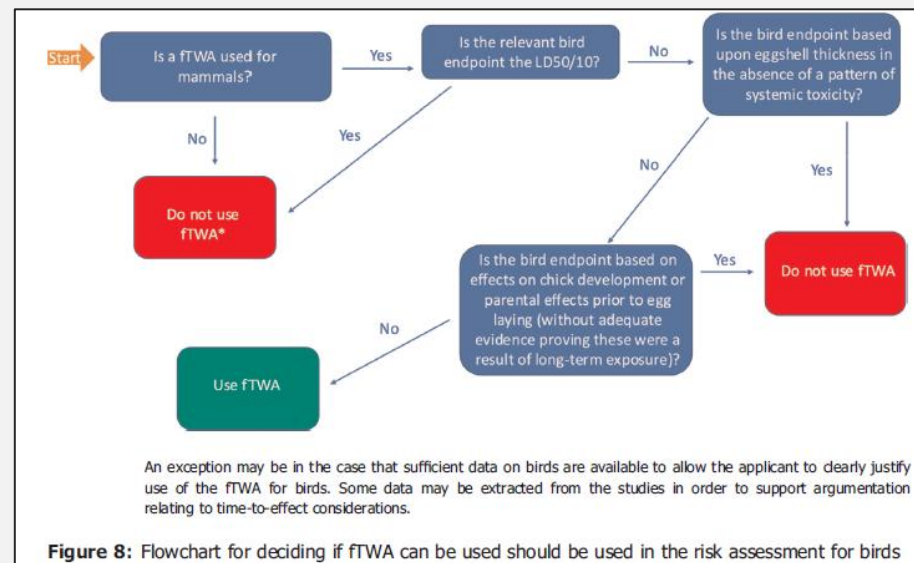
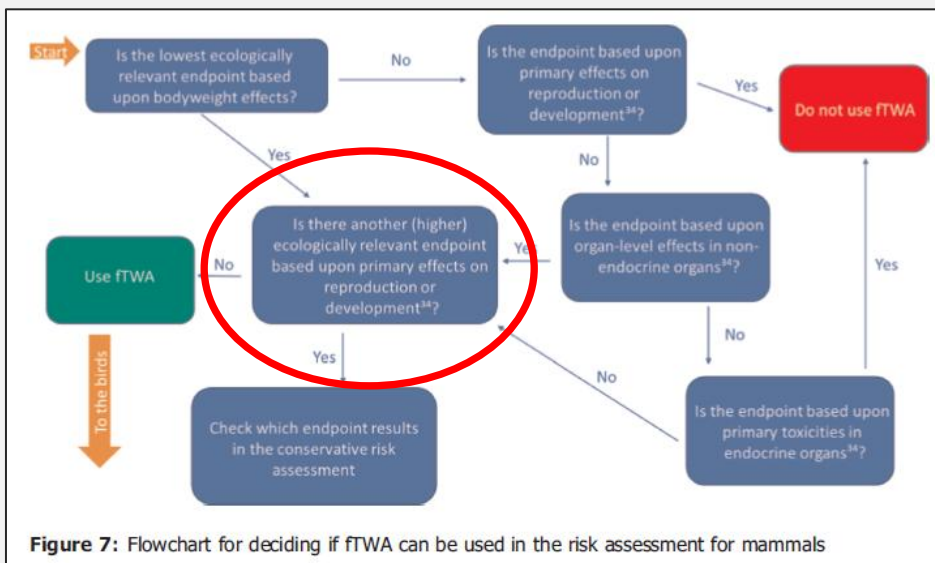
- Ambiguities, contradictions in the guidance document
- Supplemented by notes, examples and caveats
- Our interpretation of ambiguous parts
- Our proposals

Discuss possible ways to interpret the ambiguous sections of guidance.

- General ambiguities
- Ambiguities specific to birds or mammals

General points - 3x higher EP

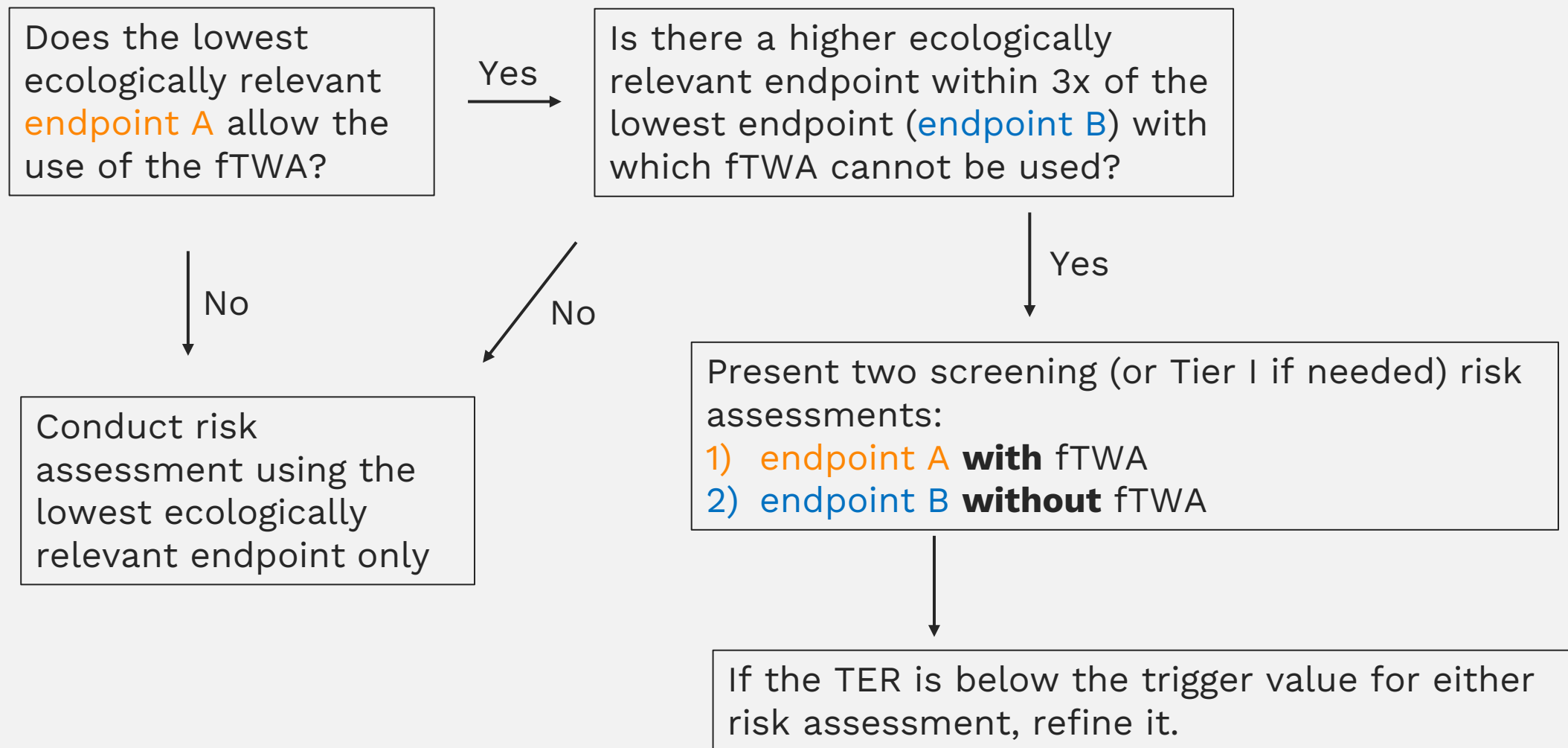
If the lowest ecologically relevant endpoint allows the use of the fTWA, it should also be checked whether a higher ecologically relevant endpoint (within 3x of the lowest endpoint) would not be appropriate with the fTWA, and if so, in that case a risk assessment should be presented using each endpoint. (EFSA 2023, page 62)



Ambiguity: According to the text, it should be checked whether there is a $\leq 3x$ higher ecologically relevant endpoint that does not justify the use of fTWA. However, this criterion exists only in the mammals flowchart.

Interpretation: This criterion should also be checked for birds.

General points - 3x higher EP



General points - mixtures

Section 12.3.1: Default approach

Combitox calculation approach whenever

- **experimental studies with the formulated mixture** are **not available**
- experiments with the formulated mixture **are available, but they do not indicate any synergism** (MDR<3)



Note that this approach does not pose any limitation in terms of refinement for the TER calculation of single substances, as the integration at the mixture level only occurs after the individual TERs have been calculated. (EFSA 2023, page 144)

General points - mixtures

Section 12.3.2: Data informed approach

Combi tox calculation approach when **formulation studies are available** for birds or mammals

$0.33 < \text{MDR} < 3$: Dose addition paradigm is confirmed

$\text{MDR} < 0.33$: Antagonistic effects cannot be excluded



If **$\text{MDR} < 3$** , DA is either confirmed ($0.33 < \text{MDR} < 3$) or antagonistic effects cannot be excluded ($\text{MDR} < 0.33$). In both cases it is considered sufficiently conservative to base the risk assessment on additive toxicity and the default approach illustrated above (see 12.3.1) should be followed. (EFSA 2023, page 144)

- no restriction on fTWA use for active substances for which it is applicable

General points - mixtures

MDR > 3: the mixture is proven more toxic than predicted by the dose addition model and synergism cannot be excluded



In the case that one of the substances in the mixture does not meet the fTWA criteria described in Section 6.1.4, then the mixture cannot be assumed to meet the fTWA criteria and dissipation for any component cannot be accounted for. (EFSA 2023, page 145)

Caveat: Approach reasonable for real synergism only. But it should be checked whether there is an explanation for $MDR > 3$ other than synergism, i.e. $LD_{50} > \text{highest tested doses}$ for both active substances and product or a co-formulant with high toxicity.

General points - mixtures

Formulation that contains
active substances:

X fTWA is allowed (effects due to long-term exposure)

Y fTWA is not allowed (effects due to short-term exposure)

No product toxicity data are
available or
 $MDR < 3$

$MDR > 3$
(**real synergism only**)

effects of active substance X are
accelerated / enhanced by
substance Y.

Risk assessment for:

X with fTWA
Y without fTWA
the formulation
(combination toxicity)

Risk assessment for:

X without fTWA
Y without fTWA
the formulation
(combination toxicity)

Mammals - pup body weight

Pup body weight reduction

It is a reduction in body weight and therefore an effect that allows the use of fTWA?

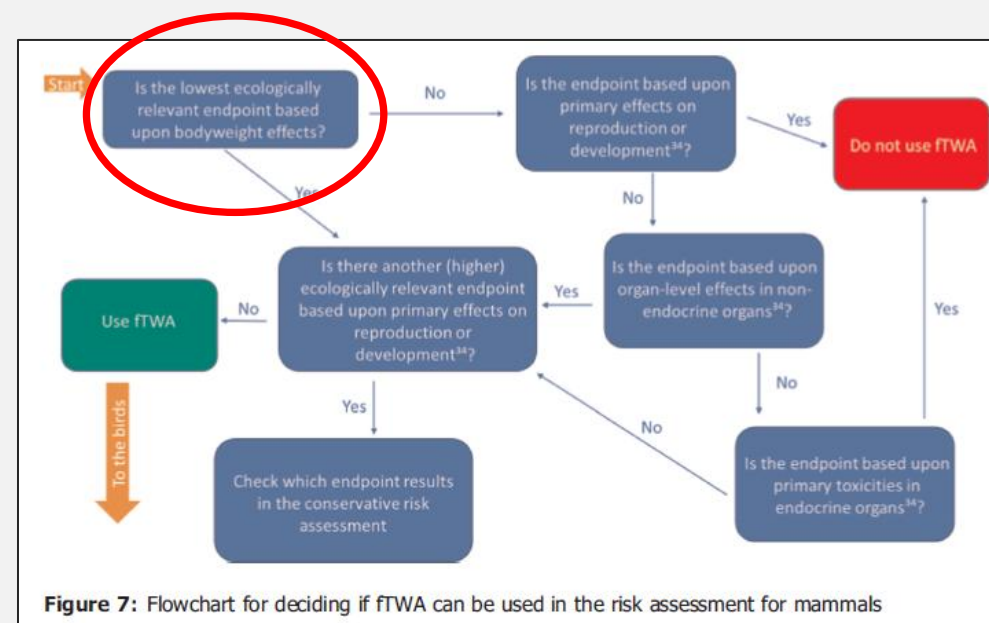
Table 10: Examples of endpoints for which the fTWA is or is not appropriate*

	Mammals	Birds
Effects for which the fTWA is appropriate	Body weight and body weight change	Effects other than those specified in the following rows of this column
	Food intake	
	Liver and kidney effects	
	Other organ-level effects (See Section 5.2.6.5)	

Ambiguity: It is not clear in Table 10 and the flowchart whether effects on body weight also include effects on pup body weight.

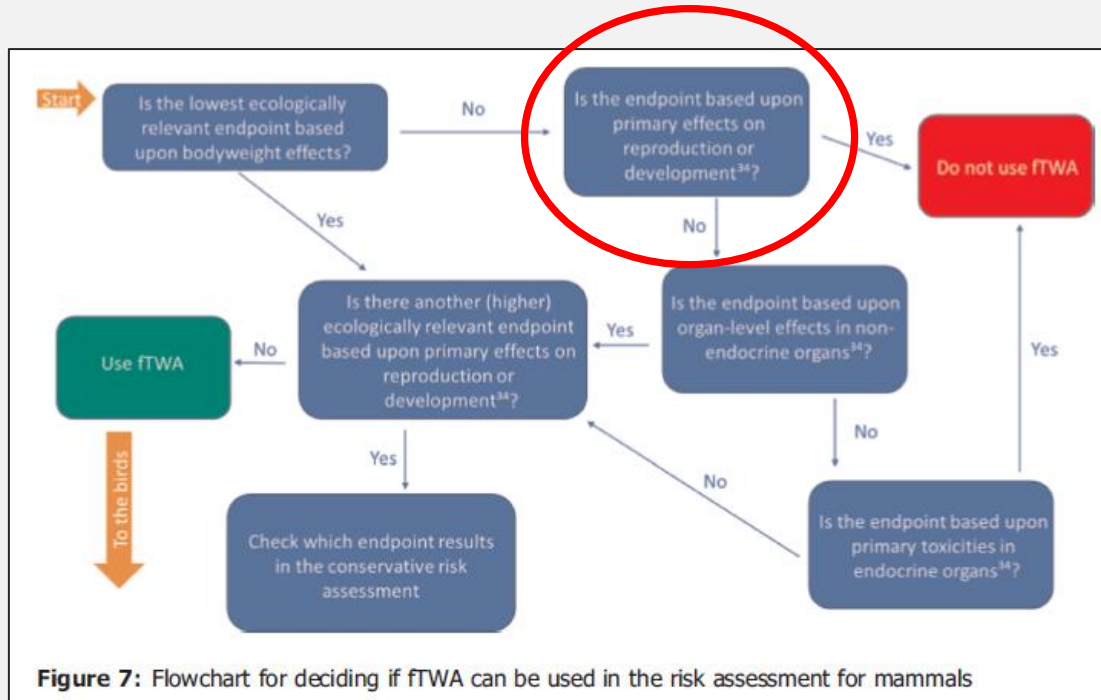
Example: Reduction of pup body weight during the lactation period

Proposal: This may be a cumulative effect and thus qualifies for fTWA.



Mammals - primary vs secondary

Are the effects observed in the mammalian toxicological studies primary or secondary (due to maternal/systemic toxicity)?



It is important to distinguish between:

- **direct toxic effects** on reproduction or on the developing fetus and
- Indirect, **secondary effects** on reproduction or development caused by the disruption of maternal homeostasis and well being

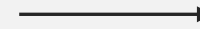
There is no clear definition of primary and secondary effects in EFSA 2023

Mammals - primary vs secondary

Are the effects observed in the mammalian toxicological studies primary or secondary (due to maternal/systemic toxicity)?

- According to EFSA workshop (April 2023) on EFSA 2023:

If maternal toxicity is observed at the same dose or at lower doses compared to the dose at which the reproductive or developmental effects are observed,



then those effects may be considered as secondary.

EXAMPLE USE OF FTWA - MAMMALS

Substance	Effect(s) at endpoint	Criterion 1 - Endpoint based upon BW effects?	Criterion 2 - Endpoint based upon primary effects on repro/dev?	Criterion 3 - Endpoint based upon organ level effects in non-ED organs?	Criterion 4 - Endpoint based upon primary toxicities in ED organs?	Criteria 5 - Is there another (higher) ecologically relevant endpoint based upon primary effects on repro or dev?	Conclusion - Can the FTWA be used?
Catcha	body weight reduction across studies and generations	yes	Not applicable - go to 5	Not applicable - go to 5	Not applicable - go to 5	No - dev effects observed in devtox studies secondary to dam effects	Yes
Tiger	Decreased gestation length and post implantation survival	No	No - effects are observed in presence of significant reduction in bw gain in the parent and reduced food consumption	No	No	No	Yes



Mammals - primary vs secondary

Are the effects observed in the mammalian toxicological studies primary or secondary (due to maternal/systemic toxicity)?

- In principle, this question is addressed in the human health assessment.

Proposal: Only in cases for which primary/secondary is still under discussion in the human toxicology section, it should be evaluated from an ecotox perspective.

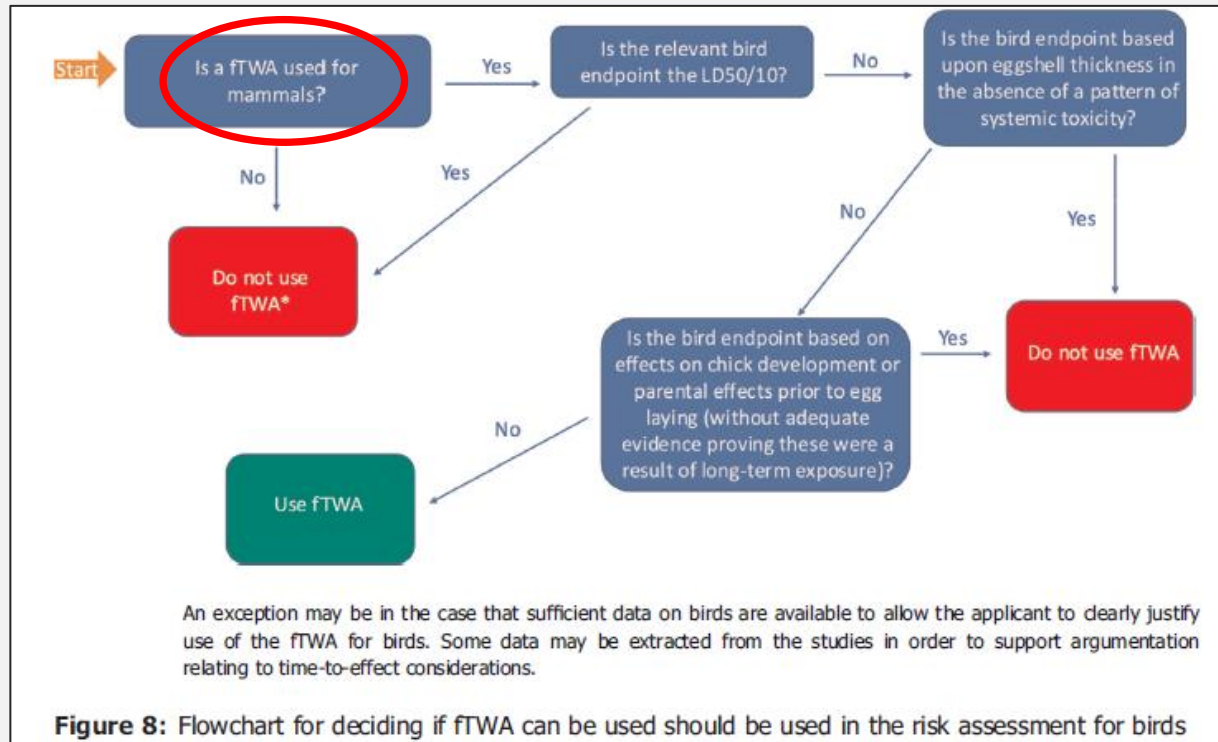
- Maternal toxicity can include e.g. effects on body weight, body weight gain, food consumption and clinical signs.

Note: A maternal effect does not need to reach population relevance to cause secondary effects on reproduction and/or development.

Note: Even primary effects can be due to long-term exposure and in that case the use of fTWA should not be excluded (see non modelling and modelling lines of evidence).

Birds - “fTWA no” for mammals

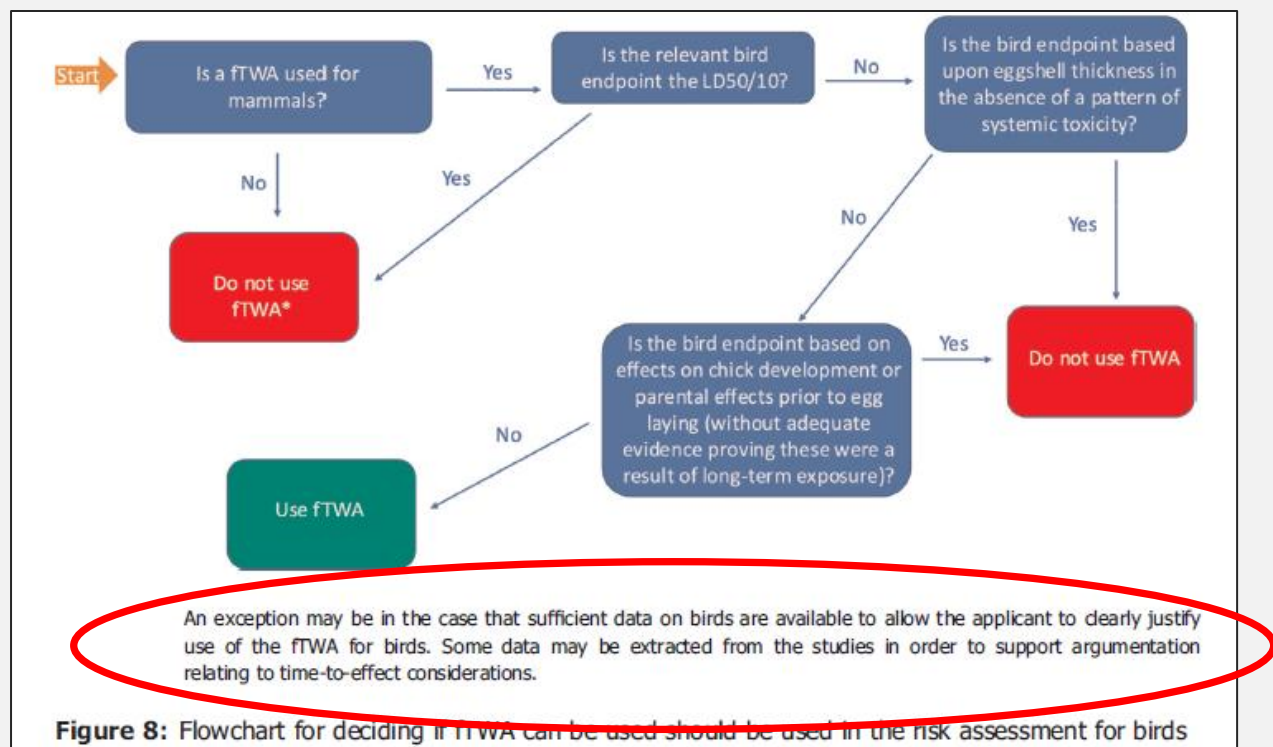
effects prior to egg laying), it is not acceptable to use an fTWA. When there are no effects observed in the reproductive toxicity test, the use of the fTWA is justified. (EFSA 2023, page 62)



Ambiguity: While the text says that fTWA can be used for birds when there are no effects in the bird reproduction studies, this is not mentioned in the fTWA flowchart for birds

Interpretation: In cases where there is an “fTWA: no” for mammals but no effects are observed in bird reproductive studies then it is an “fTWA: yes” for birds

Birds - “fTWA no” for mammals



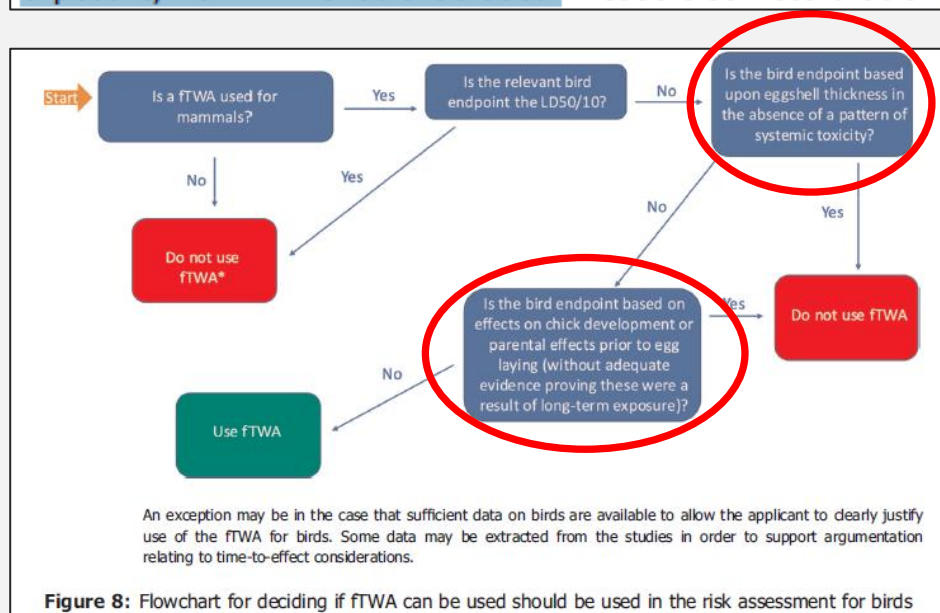
In cases where there is an “fTWA: no” for mammals and there are effects observed in bird studies, there could still be lines of evidence for fTWA applicability in birds (see on modelling and modelling lines of evidence)

Birds - parental vs maternal

more difficult primarily owing to the limited data requested for birds. As a conservative assumption, if the lowest relevant endpoint is based upon effects on eggshell thickness in the absence of systemic toxicity (e.g. significant effects on adult body weight, activity, clinical signs such as feather loss), no fTWA should be used. Due to the fewer data and endpoints available for birds compared to mammals,

studies in order to support argumentation relating to time-to-effect considerations. Effects on chick development which are not linked to parental toxicity should be considered carefully and unless significant evidence is provided to indicate that these effects are definitely linked to a long-term exposure, no fTWA should be used. Please also note that any of these effects could be as a result of

(EFSA 2023, page 62)



Effects for which case-by-case expert judgement should be employed

(Table 10, EFSA 2023, page 63)

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)

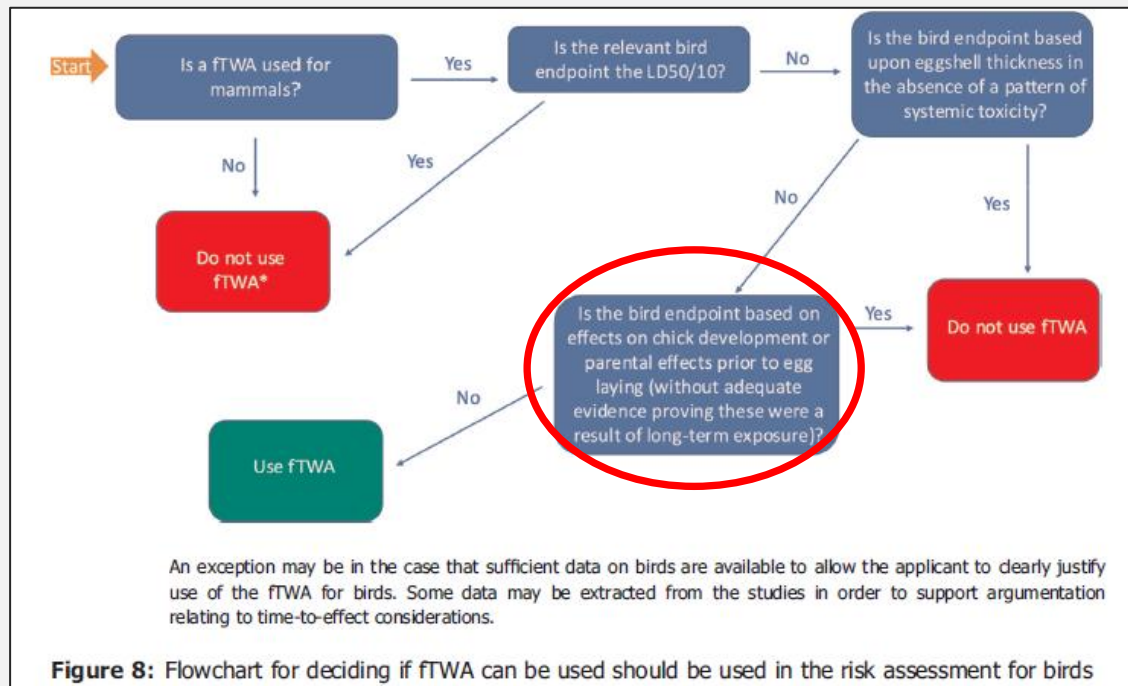
Ambiguity: Inconsistency in the terms used in text, flowchart and table 10. According to the text and the flowchart effects on parental animals should be considered but table 10 refers to females/maternal toxicity.

Interpretation: “Parental” is the correct term since egg quality is also linked to males.

Birds - primary vs secondary

studies in order to support argumentation relating to time-to-effect considerations. Effects on chick development which are not linked to parental toxicity should be considered carefully and unless significant evidence is provided to indicate that these effects are definitely linked to a long-term exposure, no fTWA should be used. Please also note that any of these effects could be as a result of

(EFSA 2023, page 62)



Contradiction: According to the text it should be examined whether the effects in chick development are primary or secondary, but this is not mentioned in the flowchart.

Birds - primary vs secondary

more difficult primarily owing to the limited data requested for birds. As a conservative assumption, if the lowest relevant endpoint is based upon effects on eggshell thickness in the absence of systemic toxicity (e.g. significant effects on adult body weight, activity, clinical signs such as feather loss), no fTWA should be used. Due to the fewer data and endpoints available for birds compared to mammals,

studies in order to support argumentation relating to time-to-effect considerations. Effects on chick development which are not linked to parental toxicity should be considered carefully and unless significant evidence is provided to indicate that these effects are definitely linked to a long-term exposure, no fTWA should be used. Please also note that any of these effects could be as a result of

(EFSA 2023, page 62)

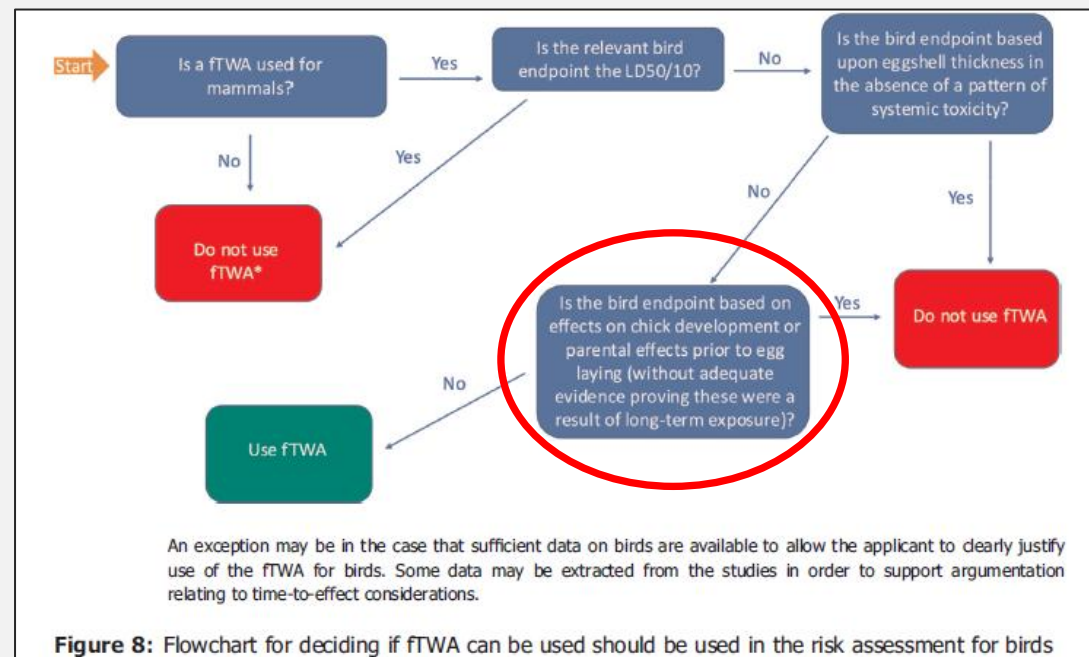
Proposal: Effects on chick development and eggshell thickness should not be considered as primary if there are significant effects on adult body weight or body weight gain, activity, or clinical signs

This proposal is based on the approach followed for mammals to decide whether effects are primary or secondary

Birds - effects prior to egg laying

However, it should also be considered that effects occurring in the parents prior to egg laying could also indicate problems with pair-forming or nesting behaviours as a result of short-term exposures. Therefore, also in these cases (where the LD₅₀/10 is used or where there is evidence of parental effects prior to egg laying), it is not acceptable to use an fTWA. When there are no effects observed in the reproductive toxicity test, the use of the fTWA is justified.

(EFSA 2023, page 62)



Contradiction: Parental effects prior to egg laying do not allow the use of fTWA. This contradicts the approach presented for mammals where the parental effects are in general considered the result of long-term exposure.

Proposal: Adult parameters are measured at multiple time points prior to the start of reproduction. Study-specific data should be used to determine whether an effect is due to long or short-term exposure.

Example (real active substance):

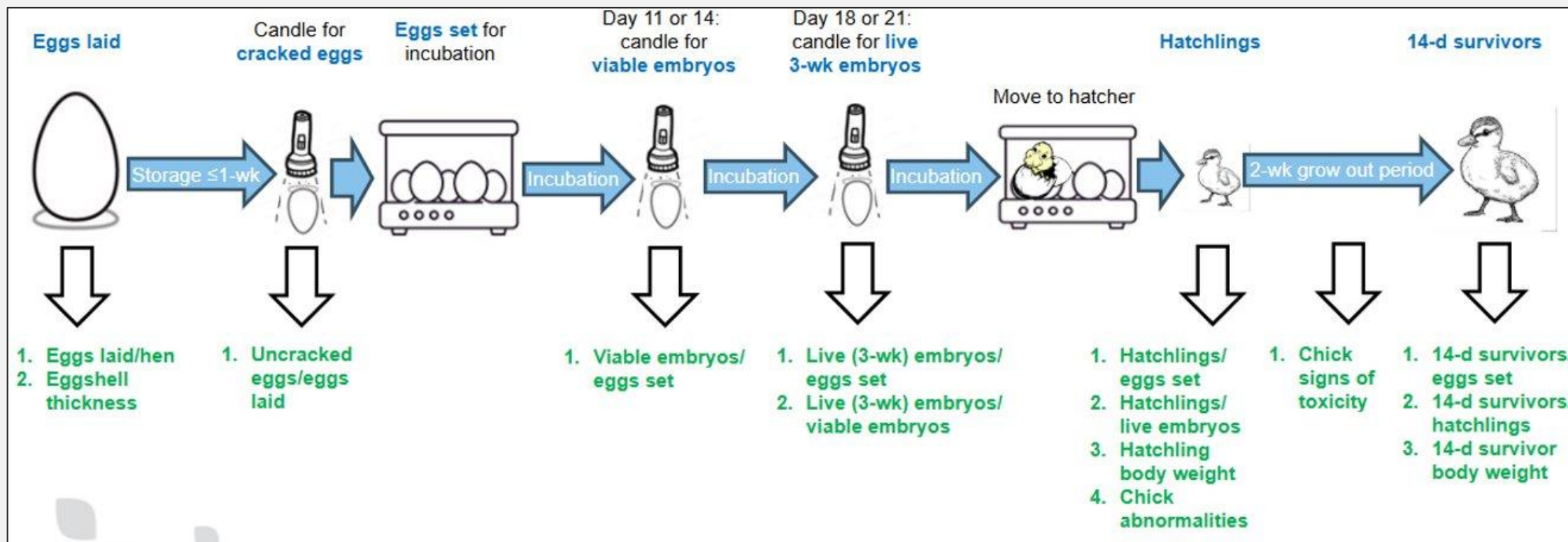
- Female body weight effects increase with exposure duration and reach $\geq 10\%$ at week 8
- 8 weeks of exposure is long-term exposure → fTWA is appropriate

Birds - reproductive studies

- In the bird reproductive studies there is ≥ 10 weeks of exposure before reproduction begins therefore, it is not possible to know if reported effects are due to long-term or short-term exposure.
- Bird reproduction studies do not measure many parameters in adults, but mammalian toxicology studies do, therefore mammalian decisions on whether effects are primary or secondary can be used as weight of evidence in fTWA evaluations for birds.
- In birds the residues transferred into the egg may be acting during a susceptible time window in chick development. However, egg residues can increase with hen exposure duration and longer periods of hen exposure may be required to reach egg concentrations that are toxic to the chick.

Birds - “chick development”

The endpoints that are considered effects on “chick development” are not defined in EFSA 2023. Many parameters are measured during the reproduction phase of bird reproduction studies:



Birds - “chick development”

There are (at least) four possible interpretations of the phrase “**effects on chick development**”:

- a) Effects in all parameters after the eggs are laid
- b) Effects observed on embryo development in eggs
- c) Effects observed in hatched chicks
- d) Developmental effects as interpreted for mammals (variations, malformations)

- Developmental effects as interpreted for mammals (variations, malformations) are often related to short term exposure



Birds - “chick development”



CLE has compiled examples from the April 2023 EFSA workshop

Bird reproduction endpoint	Active substance for which endpoint was affected	Conclusion on fTWA use for actives with endpoint affected (fTWA no, fTWA yes)
Effects on reproductive organs prior to egg laying	Meeny: ‘effects prior to egg laying (ovary regression)’	No, based on ovary regression before egg laying (criterion 4 in bird fTWA evaluation)
Eggs laid/hen	Meeny & Miney: ‘egg production’ Catcha: ‘total number of eggs laid and the number of eggs laid/female’	Meeny: No, but based on ovary regression before egg laying (egg production not listed as a reason to not use fTWA) Miney & Catcha: Yes
Eggshell thickness	Eeny: ‘eggshell thinning’	No (criterion 3 in bird fTWA evaluation)
Uncracked eggs/eggs laid	Eeny: ‘increased cracked eggs’	No, but based on eggshell thinning (cracked eggs not listed as a reason to not use fTWA)
Viable embryos/eggs set	Miney: ‘viability’ Catcha: ‘proportion of viable embryos’	Yes
Live embryos/viable embryos	Catcha: ‘proportion of viable embryos’	Yes
Hatchlings/live embryos	Catcha: ‘proportion of hatched eggs’	Yes
Hatchlings/eggs set	Catcha: ‘proportion of hatched eggs’	Yes
14-day survivors/hatchlings	Meeny: ‘14-day old survivors as a percentage of hatchlings’	No, but based on ovary regression before egg laying (14-d survivors/hatchlings not listed as a reason to not use fTWA)
14-day survivors/eggs set	Tiger: ‘reduction in almost all reproductive parameters’	Yes
14-day survivors/hen	Catcha: ‘number of chicks/female surviving 14-days’	Yes
Hatchling body weight	Moe: ‘chick body weight reduction’	Yes
14-day survivor body weight	Moe: ‘chick body weight reduction’	Yes
Chick malformations & chick signs of toxicity	Not affected in any example active substance	Uncertain, not mentioned in any of EFSA's examples

No clear answer for which parameters are “chick development”

- Based on the examples:

a) All parameters after eggs laid: **No**

b) Embryo development parameters: **No**

c) Parameters measured at & after hatching: **Unlikely**

d) Variations & malformations: **No info**

Birds - “chick development”



CLE has compiled examples from the April 2023 EFSA workshop

Bird reproduction endpoint	Active substance for which endpoint was affected	Conclusion on fTWA use for actives with endpoint affected (fTWA no, fTWA yes)
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Eggshell thickness	Eeny: ‘eggshell thinning’	No (criterion 3 in bird fTWA evaluation)
Uncracked eggs/eggs laid	Eeny: ‘increased cracked eggs’	No, but based on eggshell thinning (cracked eggs not listed as a reason to not use fTWA)
Viable embryos/eggs set	Miney: ‘viability’ Catcha: ‘proportion of viable embryos’	Yes
Live embryos/viable embryos	Catcha: ‘proportion of viable embryos’	Yes
Hatchlings/live embryos	Catcha: ‘proportion of hatched eggs’	Yes
Hatchlings/eggs set	Catcha: ‘proportion of hatched eggs’	Yes
14-day survivors/hatchlings	Meeny: ‘14-day old survivors as a percentage of hatchlings’	No, but based on ovary regression before egg laying (14-d survivors/hatchlings not listed as a reason to not use fTWA)
14-day survivors/eggs set	Tiger: ‘reduction in almost all reproductive parameters’	Yes
14-day survivors/hen	Catcha: ‘number of chicks/female surviving 14-days’	Yes
Hatchling body weight	Moe: ‘chick body weight reduction’	Yes
14-day survivor body weight	Moe: ‘chick body weight reduction’	Yes
Chick malformations & chick signs of toxicity	Not affected in any example active substance	Uncertain, not mentioned in any of EFSA's examples

Caveat: It is unclear if and how much mammalian toxicology evaluations of primary/secondary status of reproduction and/or developmental effects influenced the fTWA decisions for birds

Conclusion

In this session we have pointed out only some of the existing ambiguities related to fTWA applicability in the birds and mammals risk assessment.

- It is a challenging topic from a technical point of view, since an in-depth evaluation of data is necessary.
- The ambiguities might lead to unharmonised evaluation of data and possible delays in product registrations.
- Our view is that the fTWA applicability should be addressed at active substance level during the AIR process.
- Product submissions are approaching and there is need for an exchange with authorities.

Thank You

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