30 November-1 December 2020

Case study Ecotox

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European Food Safety Authority

T-Modality

Available evidence

Type of toxicity	Study type	Study ID in the excel
In vitro mechanistic	NVS_NR_hTRa (ToXCast)	9
In vivo mechanistic	Amphiban Metamorphosis Assay	18

T-modality

- **Is the dataset complete for the ED assessment in line with the ED GD?**

T-modality

- **Has EATS-mediated adversity been observed?**
- **Has endocrine activity been observed?**
- **Has endocrine activity been sufficiently investigated?**

Selection of relevant scenario

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected (indicate with an "x" the scenario selected based on the assessed lines of evidence)
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is no "T-mediated" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no T-mediated endocrine activity observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

Conclusion

EAS-modalities

Available evidence

Type of toxicity	Study type	Study ID in the excel
***In vitro* mechanistic**	Androgen receptor transactivation assay	**1, 25, 28**
	ATG_AR_TRANS (ToXCast)	**6**
	NVS_NR_hAR (ToXCast)	**8**
	NVS_NR_rAR (ToXCast)	**10**
	OT_AR_ARSRC1_0480 (ToXCast)	**11**
	OT_AR_ARSRC1_0960 (ToXCast)	**12**
	OT_AR_ARE_LUC_Agonist_1440 (ToXCast)	**13**
	Stably Transfected Human AR Transactivation Assay (AR STTA)	**23**
	Aromatase Assay	**4, 14, 20, 21**
	NVS_ADME_hCYP19A1 (ToXCast)	**7**
	H295R steroidogenesis assay	**24**
	ER model ToXCast	**17**
	Estrogen receptor transactivation assay	**2, 27**
	Inhibition of CYP51	**15, 22, 29**
	Steroidogenesis assay	**16**
***In vivo* mechanistic**	21-day fish assay	**26**
	Fish short-term reproduction assay	**19**
Chronic toxicity studies	Fish life cycle toxicity test	**5, 34**
	Fish early life cycle test	**33**
	Avian reproduction test	**31, 32**

EAS-modalities

- **Is the dataset complete for the ED assessment in line with the ED GD?**

Adversity

➢ Has endocrine adversity been observed?

Please describe if adversity was observed e.g. description of affected parameters for each study, concentrations where the changes were observed, % of changes, if available

EAS endocrine activity

➢ **Has endocrine activity been observed?**

Please describe if endocrine activity was observed e.g. reporting of the available evidence from in vitro data, description of affected parameters for each study, concentrations where the changes were observed, % of changes, if available, for in vivo studies

Selection of relevant scenario

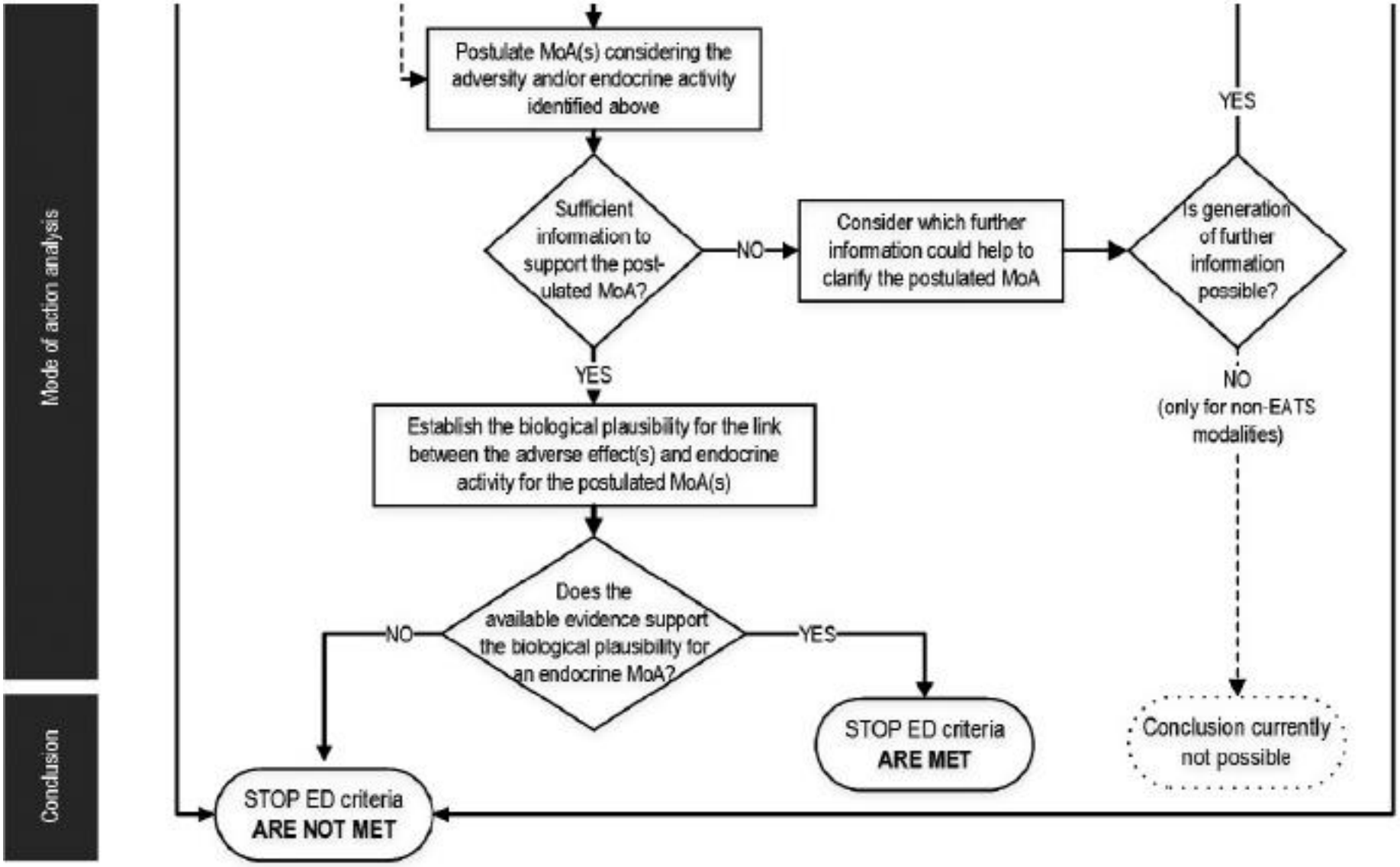
Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected (indicate with an "x" the scenario selected based on the assessed lines of evidence)
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is no "T-mediated" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no T-mediated endocrine activity observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

Conclusion

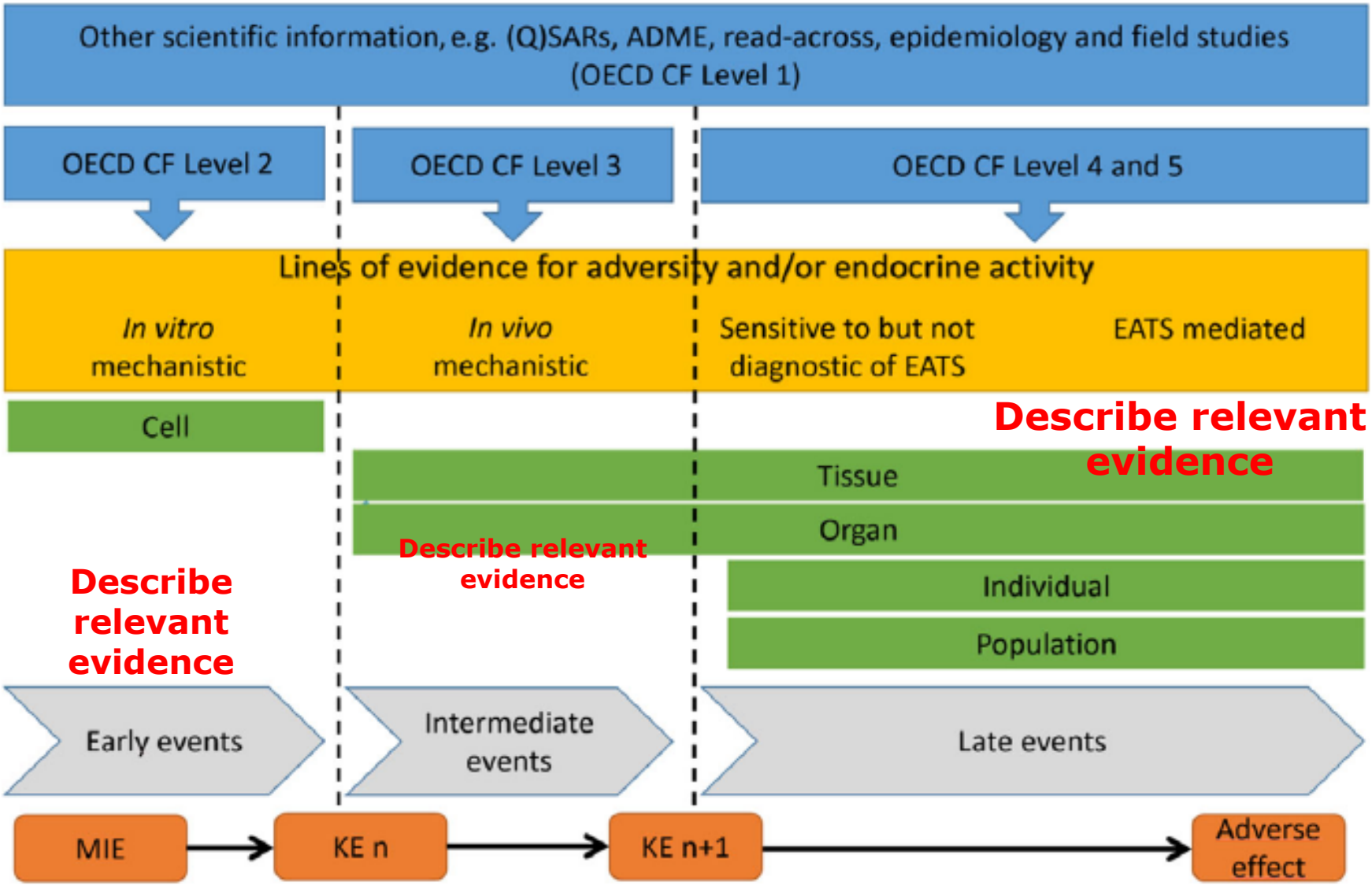
Conclusion on adversity and endocrine activity

Report overall conclusion

MoA


Mode of action analysis
Conclusion
Postulate MoA(s) considering the adversity and/or endocrine activity identified above
Sufficient information to support the post-ulated MoA?
NO
Consider which further information could help to clarify the postulated MoA
Is generation of further information possible?
YES
NO
(only for non-EATS modalities)
YES
Establish the biological plausibility for the link between the adverse effect(s) and endocrine activity for the postulated MoA(s)
Does the available evidence support the biological plausibility for an endocrine MoA?
NO
YES
STOP ED criteria ARE NOT MET
STOP ED criteria ARE MET
Conclusion currently not possible

Postulate MoA



KE: key event; MIE: molecular initiating event.

MoA

Considering the observed endocrine activity and adversity, postulate a MoA

*Please note that more than one MoA might be possible depending on the available evid*ence

MOA1 analysis

	Brief description of key event	Supporting evidence
Molecular initiating event		
KE1		
KE2		
KE3		
KE4		
Adverse effect		

Please summarise KEs and supporting evidence based on Table 6 of the ECHA/EFSA Guidance

Temporary and dose-response concordance between Kes-Table 7

Dose	KE1	KE2	KE3	KE4	AO

Please document the MoA analysis by analysing the dose and temporal concordance between KEs.

MoA1 analysis

Key event relationship

	MIE to KE1	KE1 to KE2	KE2 to KE3	KE3 to KE4	KE4 to AO
Biological plausibility for the KERs					
Empirical support for the KERs					
Essentiality					
Consistency					
Analogy					
Specificity					

Please summarise the conclusion on the biological plausibility between the adversity and endocrine activity as illustrated in Table 8 of the ECHA/EFSA Guidance

MoA1 Uncertainty and Conclusion

Identified Uncertainties	Comment

Please report uncertainties, if any, and a reason why it is considered as such

Overall conclusion

Please repeat the exercise, e.g. from slide 18 to 23 in case more than one MoA is possible

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