Potential information requirements for endocrine disruption assessment under REACH: The reliance on animal data

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Introduction

Revised information requirements to assess industrial chemicals for Endocrine Disruption (ED) have been proposed under the European Union's (EU) Registration, Evaluation, Authorisation and restriction of Chemicals (REACH) regulation. Two proposals have been put forward by the European Commission as potential options for information requirements to be added to the existing REACH regulation in order to address ED concerns. An independent review on behalf of the National Centre for the Replacement, Reduction and Refinement of animals in Research (NC3Rs) attempted to estimate the potential resource and animal use of the two policy options. This poster reviews some of the potential challenges of implementing the proposed strategies and the balance between *in vivo* testing and other approaches.

Interpretation of data requirements

- A summary of our interpretation of the testing options is given in Fig. 1.
- The options are unspecific on what *in vivo* testing is triggered by positive *in vitro* results. 'Appropriate' ED testing will likely be based on OECD GD 150, with amphibian testing for T and fish testing for EAS modalities.
- The FSDT is required instead of a FELS test if any potential ED effects are identified, though in practice there would be arguments against these being triggered on the basis of thyroid activity alone.
- At lower Annexes, it is expected that MEOGRTs would be waived on the basis that FSDTs are available, but MEOGRTs are assumed to be required at Annex X as they are a standard information requirement.

In vitro testing

- Up to 14 *in vitro* assays will be required at Annex VII including potentially up to 10 for the thyroid.
- There are currently no validated *in vitro* assays for assessing ED potential via the thyroid. There is a concern that an expectation to conduct a large number of currently unvalidated assays could lead to delays to assessing T modality, decisions made using data of equivocal quality and potentially unnecessary *in vivo* testing.
- It can take years to develop, optimise, validate and to refine interpretation of OECD technical guidance for tests.
- Even for validated methods, there are a limited number of Contract Research Organisations who can competently conduct the tests.
- The structures and properties of many industrial chemicals, e.g. salts, UVCBs, metals, are unlikely to be within the applicability domain of *in vitro* assays, which may lead to studies not being feasible.
- Mode of Action analysis may be challenging if *in vitro* data are not robust.

Figure 1. Studies required at each Annex under policy options 1 and 2.

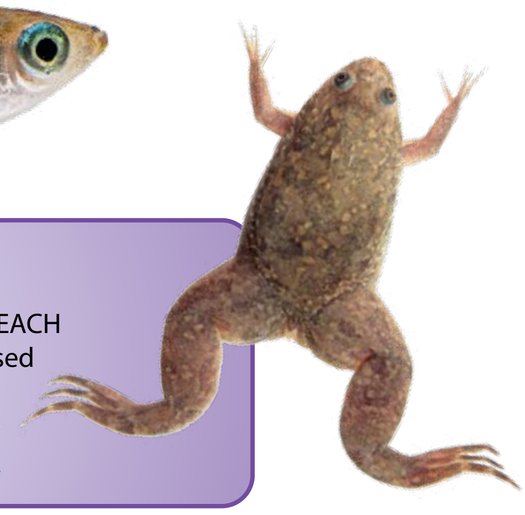
	Option 1	Option 2
	Weight of Evidence	**Assess available data**
Annex VII 1-10 tpa	***In vitro* testing** 4 assays for EAS pathways Potentially 10 assays for T pathway	***In vitro* testing** 4 assays for EAS pathways Unspecified assays for T pathway Substances with ED properties continue to Annex VIII
Annex VIII 10-100 tpa	**No specified ED testing** Substances with ED properties continue to Annex IX. If there is no human exposure, human health testing can be waived.	***In vivo* mechanistic testing** FSTRA Uterotrophic assay Hershberger assay AMA Substances with ED properties continue to Annex IX
Annex IX 100-1000 tpa	***In vivo* mechanistic testing** FSDT Uterophic assay Hershberger assay AMA	***In vivo* testing** FSDT
Annex X >1000 tpa	***In vivo* testing** M/ZEOGRT LAGDA	***In vivo* testing** M/ZEOGRT LAGDA

Read across

- ECHA's grouping approach suggests there will be an assumption that some substances have ED properties based on read across data.
- Positive results from *in vitro* tests are expected to lead to more *in vivo* testing on individual substances to discern or differentiate ED properties across a category.
- Existing read across approaches may be undermined by ED results leading to more testing on individual substances.

Replacement, reduction and refinement of animal testing

- These proposals only use *in vitro* assays as a trigger for conducting *in vivo* testing at higher Annexes if potential ED activity is identified.
- *In vitro* testing is not used to waive the conduct of any *in vivo* ED testing.
- *In vitro* results are largely disregarded at higher Annexes, which have direct *in vivo* information requirements, including ED tests on amphibians and fish.
- The only adaptation to the information requirements which does not rely on existing *in vivo* data is the ToxCast ER Bioactivity model to waive the *in vivo* uterotrophic study.



Cost and animal use

- The overall costs and animal numbers estimated for REACH testing are shown in Table 1. These estimates were based on a number of assumptions (see manuscript).
- The estimated animal use for both options is arguably unachievable considering current laboratory capacity.

Table 1. Initial animal use and cost estimates under option 1 and 2.

	Option 1	Option 2
Animal use (millions)	6.65 – 22.33	11.75 – 25.77
Cost (€ billions)	9.6 – 24.0	13.0 – 24.0

Conclusions

- In these proposals, *in vitro* studies or other non-*in vivo* data generally appear not to be used to replace, reduce or refine animal testing.
- Many of the *in vitro* tests proposed do not currently have validated methods and many industrial chemicals covered under REACH are likely to be outside the applicability domain of *in vitro* tests.
- The high number of *in vitro* methods is likely to trigger many *in vivo* tests.
- Information requirements may lead to *in vivo* testing with UVCB substances.
- Further work is required to ensure robust evaluations considering the scale of the new animal testing proposed.

Acronyms

- Organisation for Economic Cooperation and Development Guidance Document (OECD)
- Guidance Document (GD)
- Technical Guidance (TG)
- TPA: Tonnes per annum
- UVCB: Substances of unknown or variable composition, complex reaction product or of biological origin
- E: Oestrogen, A: Androgen, S: Steroidogenesis, T: Thyroid
- FELS: Fish Early Life Stage (OECD TG 210)
- FSTRA: Fish Short-Term Reproduction Assay (OECD TG 229)
- FSDT: Fish Sexual Development Test (OECD TG 234)
- AMA: Amphibian Metamorphosis Assay (OECD 231)
- M/ZEOGRT: Medaka/Zebrafish Extended One Generation Reproductive Test (OECD TG 240 / in development)
- LAGDA: Larval Amphibian Growth and Development Assay (OECD TG 241)

References

- Policy documents: http://widgixeu-library.s3.amazonaws.com/library/90010302/PolicyOptions.pdf
- OECD GD 150 - https://www.oecd.org/publications/guidance-document-on-standardised-test-guidelines-for-evaluating-chemicals-for-endocrine-disruption-2nd-edition-9789264304741-en.htm

Acknowledgement

The authors thank Defra, Corteva Agriscience and Concawe for their contributions.