Testing for Endocrine Disruption

Existing Gaps and Recommendations from a CRO Perspective

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Introduction

Testing for endocrine disruption is an important part of regulating chemical substance in the EU and USA. Accordingly, contract research organisations (CROs) face a high demand for endocrine testing. Since 2019, IES Ltd has been increasingly conducting key *in vivo* bioassays out of Level 3 or 4 of the OECD conceptual framework for testing and assessing endocrine disruptors, such as the Fish Short Term Reproduction Assay (FSTRA; OECD 229), the Amphibian Metamorphosis Assay (AMA; OECD 231) or the Fish Sexual Development Test (FSDT; OECD 234). In particular, for the registration of plant protection products, the FSTRA and AMA represent key assays to screen for endocrine disruption.

Materials & Methods

In the past years, we have been regularly conducting the FSTRA with fathead minnow (*Pimephales promelas*) and the AMA with the African clawed frog (*Xenopus laevis*) under flow-through conditions (see Fig. 1). To identify weaknesses of the current procedures, we compared our control data for all relevant endpoints with control data from the Endocrine Disruptor Screening Program published by the Environmental Protection Agency (EPA), USA.[1]

Figure 1: Flow-through facilities at IES Ltd

Results & Discussions

- **Growth and development of the control** are insufficiently addressed in the current guidelines besides a required median development stage of NF ≥ 57(AMA, see Fig. 2A)
- **The feeding regime** is essential and should be adapted and further specified (AMA)
- Additional decision criteria are needed to distinguish delayed development due to non-specific toxicity or anti-thyroidal mechanisms (AMA)
- **A uniform procedure for the performance of histopathology** is lacking even though it represents a critical endpoint in both assays (AMA & FSTRA)
- Sampling for the **VTG measurements** should be defined more in detail to decrease data variability and increase comparability between different labs (FSTRA, see Fig. 2B).
- Given that reliable toxicity data is often lacking, at least 7 and preferably a 21 days **range finder experiment** is necessary to determine a suitable concentration range (AMA & FSTRA)

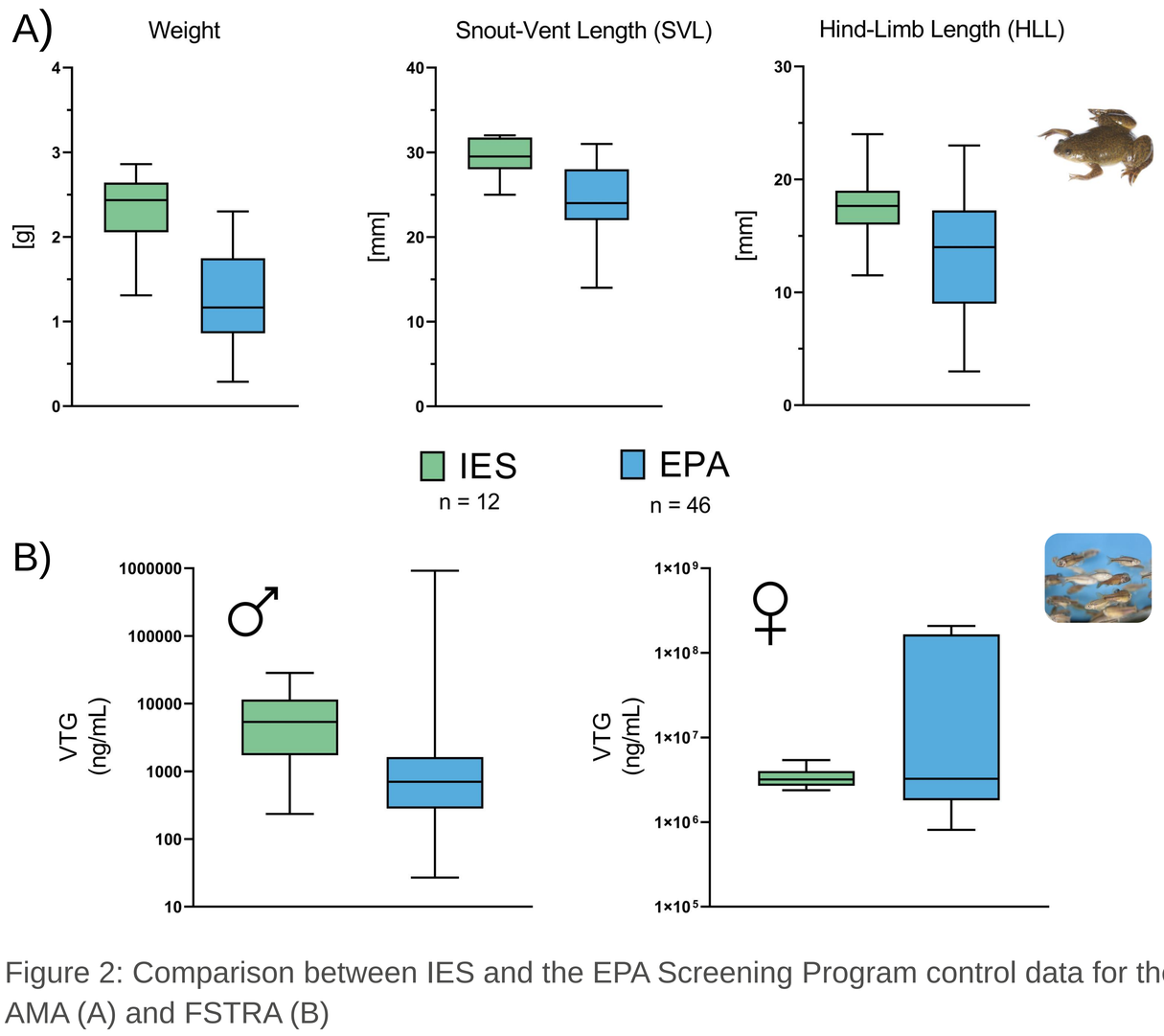

Figure 2: Comparison between IES and the EPA Screening Program control data for the AMA (A) and FSTRA (B)

Conclusion

Despite the high expertise needed to conduct the FSTRA and AMA, the guidelines should better define and describe critical steps to ensure high-quality data and prevent repetitions. We suggest all stakeholders come together to refine and revise the current procedures and develop guidance on best practices. This would improve the data acceptance and reliability and close gaps that currently leave too much room for interpretation.

If you have any questions, please do not hesitate to contact us.

References & Acknowledgement

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[1] U.S, EPA (2015). Endocrine Disruptor Screening Program Tier 1 Screening Determinations and Associated Data Evaluation Records. https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-tier-1-screening-determinations-and. (Last Updated on October 31, 2002; Last Access on November 28, 2022).