Assessing the Endocrine Disruption Potential of Biopesticides, Issues and Possible Solutions?

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Biopesticides

Biopesticides are a broad group of diverse active substances including; microbes (live and inactive), substances derived from plants, from fermentation technologies and minerals. The size of the human health and environmental safety regulatory datasets is highly variable, ranging from a dataset similar to that of a synthetic chemical to a very limited dataset, e.g. for human health assessment, acute and pathogenicity data. Biopesticides have often relied greatly on published studies, waivers and assessments based on background exposure. For many biopesticides, there is now a need to assess multiple components since biopesticides derived from plants or fermentation processes can be complex mixtures, with varying numbers of pesticidally 'active' components and impurities. Often the mixture can be an active substance, as is the case with UVCB (unknown or variable composition) substances.

Dealing with complex mixtures presents technical challenges;

- Analytical methods – identification and quantification of a sufficient proportion of the technical material
- Studies that conventionally need radiolabelled test material which may become impractical, *e.g*. absorption, distribution, metabolism and excretion data, dermal absorption studies
- Variability batch-to-batch can lead to high maximum levels of components in the speciation, which must be justified.

With the publication of Commission guidance (2024) on how to deal with secondary metabolites, living microorganisms face similar challenges in the evaluation of their secondary metabolites. This guidance sets out a detailed series of stages and steps, Stage 2 being a gathering of information which can result in a list of, potentially, dozens of components which need to be considered for their potential hazard.

A 'light' touch?

For over 20 years, there have been discussions around the concept of a 'light' touch for regulation of biopesticides and the 'Farm to Fork' strategy indicated the possibility for faster authorisation of these substances (microorganisms vs. synthetic chemicals). It is being assumed that this strategy may help increase the availability and access to low-risk plant protection products for use in the EU.

Impact of the ED Cut-off Criteria

The current regulatory focus on endocrine disruption (ED) means that a 'light' touch can be no longer considered. Many will know the phrase *dosis sola facit venenum* ('the dose makes the poison'), which underlies the Risk = Hazard x Exposure paradigm. This, however, is not the case for endocrine disruptors in the EU policy framework, where the legislation on endocrine disrupting properties has a hazard-based "cut-off" criterion *i.e.* the focus is on the intrinsic property of the substance without consideration of the relevance of potential or negligible exposure to humans or the environment.

Thereby, if a substance is considered an ED under EU Criteria, this means no or highly restricted approval. In particular, effects on the thyroid are under scrutiny, due to the known effects of disturbances of thyroid hormone levels during pregnancy. These effects can result in cognitive, learning and memory deficits, hearing loss, visual disfunction and cardiovascular impairments.

Commission Regulation (EU) 2018/605 states an active substance, safener or synergist shall be considered as endocrine disrupting, if it meets all of the following criteria:

i. It shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;
ii. It has an endocrine mode of action, *i.e.* it alters the function(s) of the endocrine system; and
iii. The adverse effect is a consequence of the endocrine mode of action.

Meeting these criteria effectively means that an active substance will not be approved in the EU under Regulation (EC) No 1107/2009.

Addressing the ED properties of a substance is undertaken by following EFSA/ECHA Guidance (2018) requiring four endocrine modalities to be addressed; Estrogen, Androgen, Thyroid and Steroidogenesis (EATS). For synthetic chemicals, the standard dataset includes a range of studies; subacute, subchronic, lifetime, reproductive and developmental studies, which comprehensively address these modalities. However relatively often, because of some evidence of ED adversity, further mechanistic data are still requested, which can be costly. An *in vitro* dataset examining the EAS modalities costs *ca.* £50-60,000, with any positive results triggering OECD Level 3 studies such as the *in vivo* uterotrophic bioassay in rodents (*ca.* £50,000) and/or the *in vivo* Hershberger assay in rats (*ca.* £60,000).

If any effects indicative of T-Modality adversity (effects on thyroid weights, histopathological changes or effects on thyroid hormone levels) are seen, it can be even more costly to address this modality.

The T-Modality

In rodents treated with an active substance, adaptive effects can be seen on the liver as it responds to the exposure. This is an evolutionary response which enhances the metabolism and excretion of what is a foreign substance to the rodent. However, when liver metabolism is induced, a secondary effect can occur on thyroid hormones. These too can be metabolised and excreted more rapidly as a secondary effect of the induced liver, and this can cause a drop in circulating thyroid hormones to which the rodent responds by producing more thyroid hormones. This can cause increases in thyroid weights and histopathological changes, *e.g.* thyroid cell hyperplasia. In 2019, EFSA published a paper which indicated almost half of the 128 pesticides investigated had a liver mode of action. It is not surprising then that most active substances causing effects on the T-modality investigate a liver-based mode of action to try to exclude human relevance. Such a package of studies can cost several £100,000 and if there are measured changes in hormone levels this can trigger the need for a Developmental Neurotoxicity study (DNT) or a Comparative thyroid assay (CTA) which may cost *ca.* £800,000.

Testing with non-target organisms

Investigation of endocrine disruption is not limited to potential ED effects on humans (which are addressed *via* mammalian toxicology testing), but also on other vertebrate non-target organisms (NTOs) such as wildlife birds and mammals, fish and amphibians. Following the EFSA/ECHA Guidance (2018), a standard number of studies is also usually required (aquatic testing) as a minimum to examine whether the substance may exhibit EATS modalities related activities as a first step.

These studies are conducted in flow-through systems and there are various practical issues related to them when dealing with multicomponent substances, for which focus cannot be given on only one component, since there is no definition of a single active substance within the mixture. Such practical issues include:

- Water solubility of the mixture itself and/or differences in it within the various components.
- Maintenance and analytical recovery of all components in water system, *i.e.*
  i. Differences in individual water solubility
  ii. Degradability
  iii.Potential to lose material due to adsorption to glassware
- Maximum Tolerable Concentration (MTC) setting is key to successful aquatic endocrine testing as there are only three test concentrations which need to be in an area where no systemic toxicity is triggered (masking ED related effects when these are observed) but high enough to satisfy the regulators that such levels are sufficient to trigger ED activity. For multicomponent substances, usually of low systemic toxicity to *e.g.* fish, setting the MTC is a challenge.
- Interpretation of results: if studies exhibit no ED activity, the challenge would be to properly interpret the results in the context of the presence of all components in the test system. Repetition of vertebrate studies is not a viable option, and as such, these are studies to be performed successfully at the first attempt.

Conclusions

ED potential has to be examined in both the human health and NTO contexts, and ERM use an approach which integrates these disciplines; designed to avoid vertebrate testing and optimise costs. As there are no published or established testing strategies to assess for ED with complex mixtures/secondary metabolites, we employ a stepwise screening approach using strategies including;

i. OECD Level 1 data: phys-chem, literature search, natural occurrence, presence in food & feed, US ToxCast, QSAR (OECD Toolbox, Danish QSAR databases)
ii. Activity studies *in vitro* and other non-vertebrate testing (XETA, RADAR, REACTIV)
iii. Combining the above in a weight of evidence analysis with all existing mammalian toxicity data

A challenge remains around whether regulators would accept such approaches given their need to remove uncertainty from the assessment.

Figure 1.
Chromatogram of a complex mixture

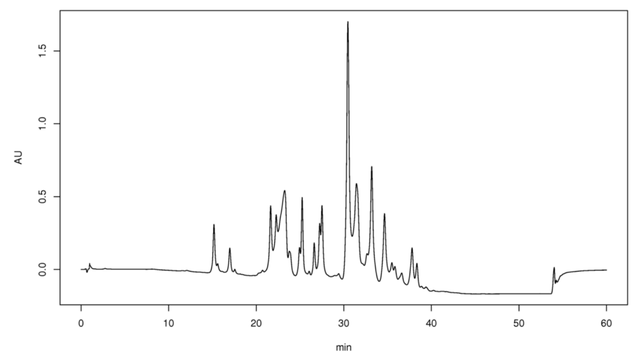


Figure 2.
Stage 2 of the EFSA Secondary metabolites guidance

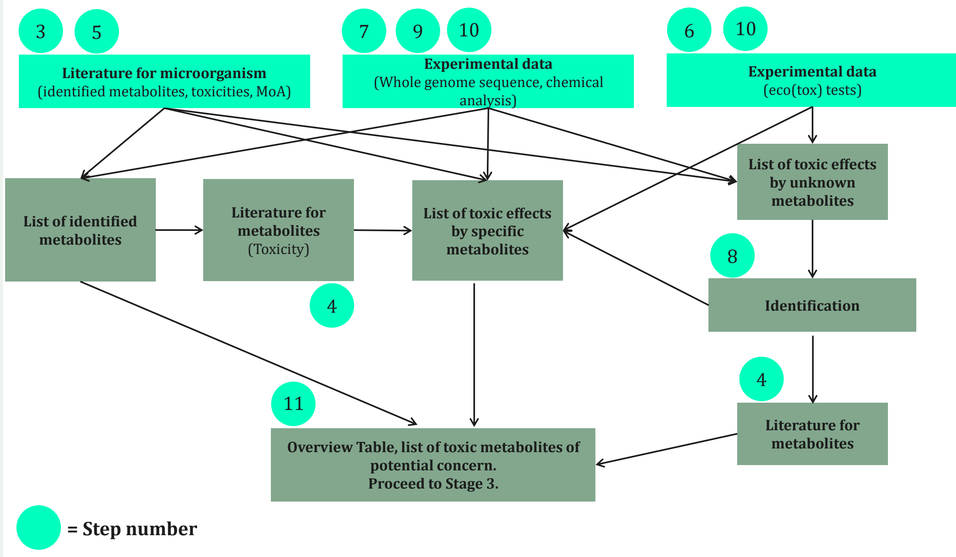


Figure 3.
ED Cut-off Criteria

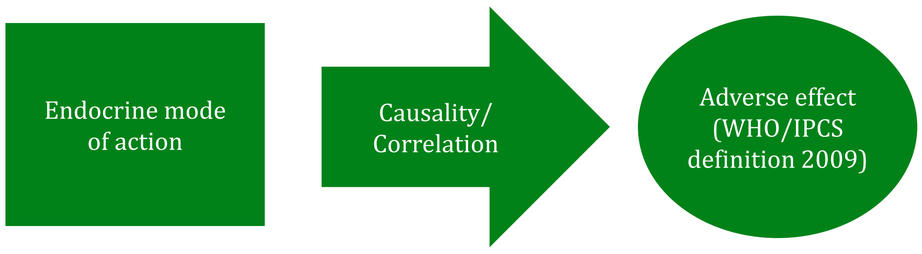


Figure 4.
Regulation of thyroid hormones

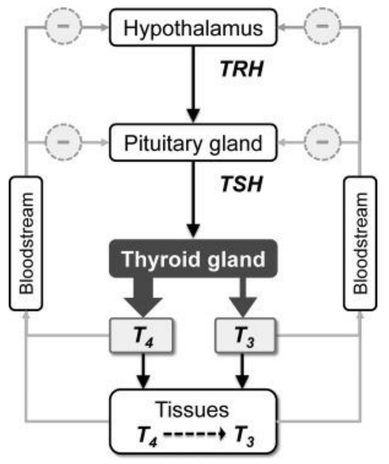
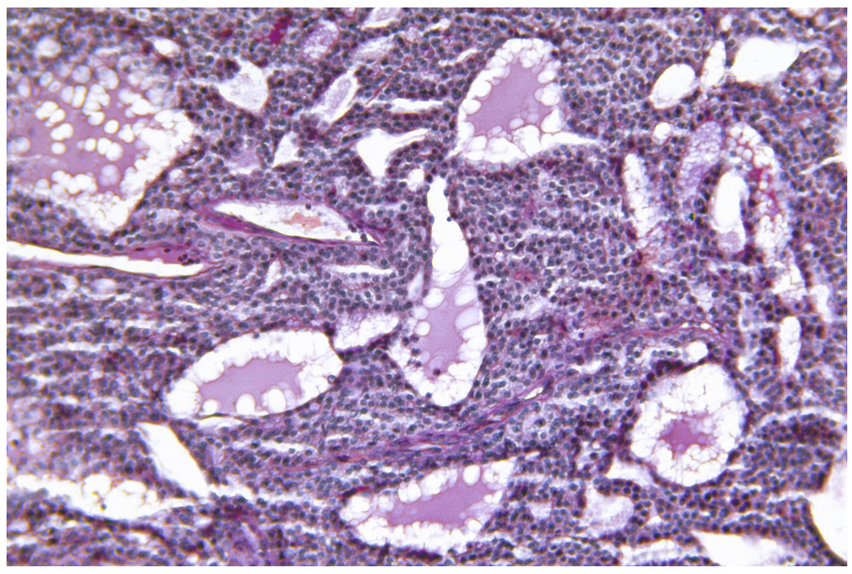


Figure 5.
Thyroid Cell hyperplasia



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