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EFSA new guidance on endocrine disruptors: comments, critical aspects and a case study

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KEY WORDS: Endocrine Disruptors, Pesticide

HIGHLIGHTS:

- ✓ The EFSA ED guidance [1] has been developed to help assessors of the regulatory authorities on the definition of the scientific criteria for the determination of endocrine-disrupting properties in the context of Regulations (EU) No 16 528/2012 and (EC) No 1107/2009. It mainly illustrates the steps required to recognize a substance as an endocrine disruptor and deals with effects caused by Estrogen, Androgen, Thyroid and Steroidogenesis (EATS) pathways as there is relatively large mechanistic knowledge of the several key events and standardized test guidelines available for in vivo and in vitro testing.
- ✓ The overall objective resulting from this project was the compilation of a specific Database for a pesticide active substance and its metabolites, which is comprising all available parameters that are considered relevant when investigating the ED properties.
- ✓ The database represents a practical tool to help assessors in assessing and analyze the evidence for endocrine disrupting properties.
- ✓ A useful-friendly client-side form of the database was created. All the important information were inserted in one unique form, with simple on-click buttons to interact the different data structures. Collection of data from substance X dossier represented a new exercise related to new approach for evaluating ED properties of active substances.

Definition: **"An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (WHO/IPCS definition of an endocrine disruptor - 2002). [2,3]**

ED CRITERIA:

All toxicological relevant information must be considered:
- ✓ guideline studies
- ✓ other scientific data selected through systematic review

All collected studies should undergo quality assessment to establish data reliability [5, 6]

Sufficient information available on the ED properties ?
- ✓ conclude that the ED criteria are not met; or
- ✓ start the mode of action analysis (MoA)

Is there any adverse effect caused by an endocrine mode of action? If the answer is no a conclusion could be drown that endocrine criteria are not met; otherwise if the answer is yes: a mode of action should be postulate, additional info must be generated

No biological plausibility between endocrine activity and adverse effect, the substance is considered not to meet the ED criteria

MoA analysis supports the link between adverse effects and endocrine activity, the substance is considered to meet the ED criteria.

The last step is the final conclusion on the endocrine properties of the substance

- **it shows an adverse effect in an intact organism or its progeny, , which is a change in the morphology, physiology, … that results in an impairment of functional capacity….**
- **it has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system.**
- **the adverse effect is a consequence of the endocrine mode of action**

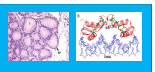
Gather Information → Assess the evidence → Initial analysis of the evidence → Mode of Action Analysis → Conclusion on the ED criteria

Grouping parameters OECD conceptual framework:

- In vitro mechanistic — *OECD lvl 2: data and parameters from mechanistic in vitro data such as estrogen receptor binding assay.*
- In vivo mechanistic — *OECD lvl 3: information from in vivo mechanicistic studies such as uterotrophic assay*
- EATS-mediated — *OECD lvl 4: in vivo parameters linked to adverse endocrine effects such as endocrine organ weight and endocrine organ histopathology.*
  OECD lvl 5: in vivo parameters not directly linked to a possible endocrine adverse effect but that should be taken into consideration.
- Sensitive to, but not diagnostic of EATS information on potentially adverse effects that cannot be considered (exclusively) diagnostic of any one of the EATS modalities
  OECD lvl 4 & 5 [4]

Data are assembled into lines of evidence based
- ✓ Lines of evidence for adversity
- ✓ Lines of evidence for endocrine activity

Assess the evidence by assembling all information into different evidence line: one for adversity of endocrine parameters from in vivo studies (such as uterus weight or testes histopathology) and one for endocrine activity from mechanistic studies (such as receptor binding)

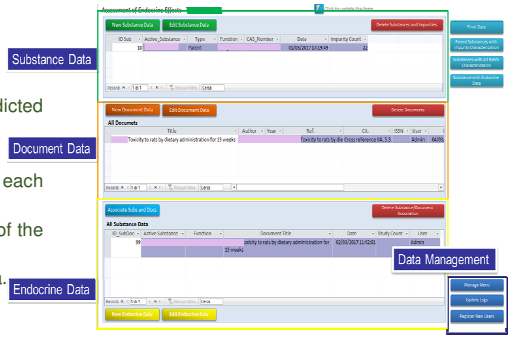


If adverse effects and/or endocrine activity are identified, postulate MoA(s) using the available lines of evidence (adversity and endocrine activity)

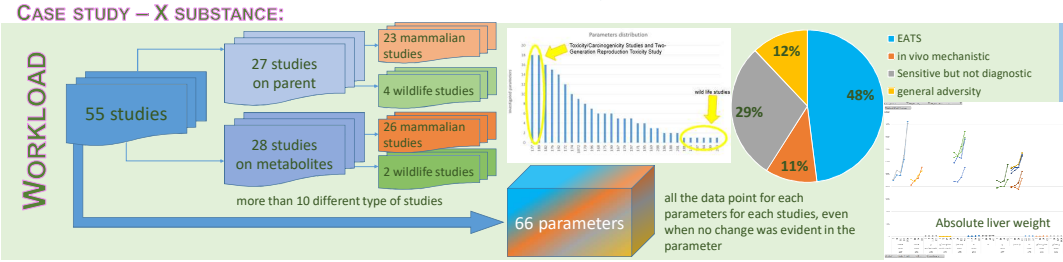
The mode of action should be built on the basis of available mechanistic information already present in the data package or retrieved from the literature. [7]

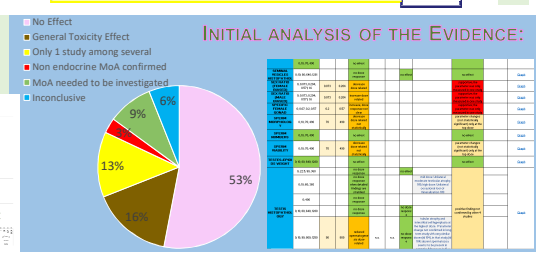
THE DATABASE:

- ✓ The Microsoft Access database was selected as suitable for this specific data collection due to the number of predicted records to be stored in and the ability of the toxicologist to work with this specific platform.
- ✓ Two coupled databases: server-side and client-side.
- ✓ Three main tables: physical-chemical properties; study details; all the dose-response data for each parameter in each study.
- ✓ Most of the field were constrained with menu combo boxes and several queries were prepared to check the quality of the inserted data to facilitate the data entry and to limit typos → Attention to the users' needs.
- ✓ Completely and directly compatible with the EFSA excel form proposed in the guidelines for collecting endocrine data.
- ✓ Pivot graphs: graphical picture of all the dose response curves → Possibility to conclude at a glance.



CASE STUDY – X SUBSTANCE:





COMMENTS ON CRITICAL ASPECTS OF THE ENDOCRINE DISRUPTOR GUIDELINES:

List of critical aspects of the EFSA ED guidelines that, even if without a practical solution, could offer several points of thoughts and underlines the need of further strategies to be planned.

- ✓ Systematic Review: If few scientific articles on active substance itself are available, could the assessors rely on similar compound throughout read-across approach? On the other hand, how to face substances with a lot of published data? The assessment could be very challenging and time consuming. Available open literature data (in-vitro, animal studies and epidemiological studies) are often not detailed enough to conclude on reliability of the data. How to weight up this type of information in the context of the ED assessment?
- ✓ Assessing the evidences: Both positive and negative results should be recorded. This could led to thousands of lines that will have to be inserted in the data sheet since "no effects" in all organs, tissues and apparatus will have to be scrutinized starting from original data reports. Consequently a variable time-consuming and resources-consuming workload should be anticipated, depending on the amount of data.
- ✓ Postulate a MoA: If the lines of evidence for adversity or for endocrine mechanism are somehow positive for some parameters, a mode of action should be supposed, built and corroborated by information and data retrieved. Mode of action hypothesis is a very challenging procedure that needs a lot of expert judgement. This could lead to large differences in the assessment and different conclusion.
- ✓ Inconclusive cases: Actually it is true that evidence of absence of an effect is more robust that the absence of the evidence of an effect. However, could really a change, for example, in utero weight be sufficient for labelling a substance as endocrine disrupter? The guideline does not list any conclusion different from endocrine criteria met or endocrine criteria not met. It seems that too many evidences are need to conclude that a substance is not an endocrine disruptors and very few evidence are sufficient for the positive conclusion.

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