Public consultation on EDC guidance document

Response from the Health and Environment Alliance (HEAL)

HEAL
Promoting environmental policy that contributes to good health

31 JANUARY 2018

GENERAL COMMENTS

In general, it is encouraging to see that many of our concerns about the previous version were addressed in the updated version of the guidance document. We especially welcome the acknowledgment of possible non-monotonic dose-responses (NMDR) as well as statements with regards to testing at sufficiently high doses (in order to avoid low dose testing), which we find important improvements. **However, it should be noted that this is not in concordance with testing guidelines (TGs).** For example, in the EOGRTS it is stated that the highest dose tested should give "some toxicity". This is neither specified nor defined. In practice, EOGRTS studies are increasingly performed with doses well below the effect doses. It should be acknowledged in the guidance document that for ED identification, testing should be performed with sufficiently high doses and statistical power to allow assessment of adverse effects (rather than giving statements about maximal doses).

REMAINING POINTS OF CONCERNS

1. **The guidance document is aimed at identification of an ED based on EATS-mediated properties in mammals**. Contrary to what the title of the document suggests and what is stated all throughout the document, in its current state the guidance document IS NOT reaching the objective of facilitating the identification of endocrine disruptors in the context of regulations 528/2012 and 1107/2009 beyond this focus. **This issue should be stressed repeatedly throughout the entire document to place more emphasis on the limitations of this guidance. We also believe that the title should also be adjusted to reflect this narrow focus** (we suggest "Guidance on identification of EDs with EATS properties in mammals"). Not only does the EATS-mediated focus leave much room for false-negative conclusions, but there currently also is no placeholder on how to expand the guidance beyond EATS-mediated properties and ED-related endpoints of concern that are currently not addressed in existing TGs. Clearly, this is not reflecting the ongoing progress in scientific knowledge, foreseen amendments to existing test guidelines, and development of novel test methods.

2. **The level of proof remains a serious concern.** It not feasible to take an EOGRTS with cohorts and F2 (TG443) as the crucial decision point in order to decide on the ED modality of a substance. The regulatory requirement is a 2-generation reprotoxicity study (TG416), which clearly is less suitable to address ED-related endpoints. There is a major concern for the situations where EOGRTS (including cohorts 2/3) is not available, or if not all endpoints have been assessed (scenarios 2a ii and iii in the guidance). In this case, negative CF level 3 tests (uterotrophic/Hershberger assay) would suffice to conclude that a substance is not an ED. Also, this suggests a primary focus on ED effects in mammalians and disregards effects on non-mammalians and non-vertebrates.

3. **The demonstration of a mode of action (MoA) is not a requirement of the identification criteria themselves as adopted in the context of the pesticides (PPP) and biocide (BP) regulations.** The criteria state that the adverse effect should be the consequence of an endocrine MoA. Yet, the entire guidance document is aimed toward the demonstration of the MoA. A MoA description can help to demonstrate biological plausibility (namely address the question: can a substance lead to an adverse effect as a result of the endocrine activity of a substance?) but not be the main focus of the demonstration.

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4. **We have concerns about the part addressing the identification of ED substances via multiple MoAs.** It is not reasonable to assume that only one and linear MoA will exist. For instance, disruption of estrogen signaling will lead to multiple adverse effects, also depending on the timing of exposure. Conversely, multiple MoAs may contribute to a single adverse effect (e.g. infertility). In order for the guidance to facilitate rather than hinder identification via multiple MoAs this it should at least be acknowledged.

5. **As currently described in the guidance, biological plausibility should be established based on dose-response and temporal concordance as well as essentiality for each key event (KE).** This level of information is not provided with standard information requirements nor with existing TGs. It is not clear from the guidance document how the absence of these data will impact ED identification. Therefore, this guidance remains a scientific approach toward ED identification (with EATS properties in mammals), with seemingly limited application for regulatory purposes. In this light, it should also be stressed that ED identification is a hazard-based assessment, not a risk assessment.

6. **Finally, while we appreciate that the guidance document acknowledges several of the current limitations for ED identification, we regret that the document lacks any practical guideline on how to address these limitations and also on how new knowledge will be included in the document in the future as it expands**. While the document is a good start to help identify EDs with EATS properties in mammals, clear guidance is still missing on how to facilitate the identification of EDs with other properties in both (mammalian) non-target organisms and invertebrates. Clear language on how in practice the new scientific knowledge and advances on testing guidelines will be integrated in the document to truly expand its scope are still missing.

SPECIFIC COMMENTS

7. **Disclaimer, page 2 Lines 54-61.** Suggest to strike this paragraph. Clearly ED identification is relevant for other regulatory frameworks as well. However, the regulatory requirements vary substantially, which leads to different starting points for ED assessment. Therefore, the current guidance is a priori not applicable to other frameworks than regulations 528/2012 and 1107/2009.

8. **Page VI. Glossary of terms.** Rephrase the definition/explanation of "Biological plausibility" as follows: "In the context of this guidance, biological plausibility is considered to be the level of support for the link between the adverse effect and the endocrine modality of a chemical (PPP/BP)".

9. **Page VI. Glossary of terms.** Rephrase the definition/explanation of "Consistency" as follows: Strike "consistent observation….increase the support".

10. **Page VI. Glossary of terms.** The definition/explanation of "Essentiality" is not clear. Please clarify.

11. **Page VIII. Glossary of terms.** Strike first sentence: "Biologically plausible sequence of substance-specific key events, starting with exposure and proceeding through the interaction of the substance or its metabolites with a cell leading to an observed effect supported by robust experimental observations". We suggest to re-phrase as follows: "A MoA is the sequence of biochemical, functional or anatomical changes resulting from the exposure of a living organism to a substance".

12. **Section 2. Scope of the guidance document, Page 2. Line 189.** Please add "both underpinned by the precautionary principle" at the end of the sentence.

13. **Section 2. Scope of the guidance document, Page 2. Lines 192-196.** Please delete the sentence starting "As a consequence…" and replace by "This guidance document should only be used to facilitate the identification of endocrine disruptors with EATS properties for mammals in the context of the BP and PPP regulations".

14. **Section 3. Strategy. Page 3, Line 269-272.** The a priori questions need rephrasing, because they are confusing as stated: "are there endocrine activity and adverse effect(s) relevant for humans which can be biologically plausible linked in an endocrine MoA?" It is not clear which question needs to be answered here: the biological plausibility of the link between endocrine activity and adverse effect, OR the relevance for humans. These are two different questions. We suggest to separate and reformulate as follows:
    - Is there a biologically plausible link between the endocrine activity of a substance and the occurrence of adverse effect?

- Is the biologically plausible link between the endocrine activity of a substance and adverse effect(s) relevant for humans/non-target organisms?

15. **Section 3. Strategy. Page 3, paragraph 3. Line 277-279**. "From a regulatory point of view, a **firm conclusion on whether a substance does or does not meet** the ED criteria is always required…" This statement is not reflecting the limitations of the present guidance document: the strategy currently outlined in the guidance document is focused on EATS-parameters in mammals, it does not allow for a scenario that it is not possible to conclude on an ED modality and the guidance does not provide a placeholder to include gained scientific knowledge as well as improved (novel) test methods to better identify EDCs. Please adapt the sentence to these limitations by removing "firm", replacing "does not meet" by "is considered unlikely to meet", and also add "based on EATS-mediated parameters in mammals".

16. **Section 3. Strategy. Page4. Line 310**. Rephrase to "Expert judgement *is* necessary" (instead of "could be").

17. **Page 5. Line 314. Table 1: Please rename the title of the table to read:** "Factors that *may* be considered in the weight of evidence assessment".

18. **Section 3.1. Page 6. Line 365-376 – the assessment strategy.** The described strategy reads very evidence-heavy and we question whether this level of evidence will ever be possible to gather. Moreover, we believe that the document puts too much emphasis on EATS-mediated adversity parameters and that the absence thereof or presence of "sensitive to, but not diagnostic of EATS parameters" should not lead to the conclusion of absence or presence of ED modalities, but rather to the impossibility to conclude. The current situation, with a lack of appropriate data, limitations in test methods, and focus on EATS-mediated parameters in mammals, leads to a serious concern for false-negative conclusions. The guidance document should AT LEAST clearly acknowledge this narrow focus.

19. **Paragraph 3.1. Page 7. Line 377-384**: The implications of this paragraph in light of this guidance are unclear. As currently phrased, it reads like the GD only aims to determine effects for human health and mammals as non-target organisms. This paragraph seriously diminishes the value of for example frog/fish tests and suggests that effects on non-mammalian, non-target organisms are not important. Not only is the primary focus on human health not in line with current test strategies and regulatory frameworks, non-mammalian data are also deemed relevant for identification of human hazard. For example, the LAGDA assay could also be considered indicative for thyroid disruption in mammals. We suggest to strike this paragraph. Or, in case that the guidance actually is indeed aimed to focus on human health and mammals as non-target organisms, to clearly state this throughout the guidance, especially in the title and the disclaimer.

20. **Paragraph 3.1. Page 9. Figure 1.** Please delete the asterisk and corresponding footnote to "Have all EATS-parameters been investigated". It now reads as if EATS-parameters can only be sufficiently investigated when an EOGRTS (TG 443) is provided. Considering that this is not a mandatory test under PPP/BP regulations, this is unrealistic to use this as critical decision point in the flow chart.

21. **Page 12. lines 97-98.** We welcome the explicit statement "all mandatory studies to be carried out according to the latest version of the corresponding test guideline".

22. **Paragraph 3.2.2.1. Page 12. lines 114-120**. While we welcome the guidance on test doses, it should be noted that this is not in concordance with TGs. For example, in the EOGRTS it is stated that the highest dose tested should give "some toxicity". This is neither specified nor defined. In practice, EOGRTS studies are increasingly performed with doses well below the effect doses. This paragraph should be rewritten in a way that (rather than give statements about maximal doses) for ED identification testing should be performed with sufficiently high doses and statistical power to allow assessment of adverse effects.

23. **Paragraph 3.2.3. Page 14. line 241**: Please correct "reference error".

24. **Paragraph 3.4. Page 26. lines 376-378**. A dataset is considered sufficient to assess EATS-related endocrine activity when "all the EATS-mediated parameters foreseen to be investigated by OECD TG443 have indeed been measured and the results included in the dossier". This is not in line with the information requirements (page 116: EOGRTS may be provided as alternative for TG416 and cohorts 2/3 should be considered). Therefore, it is not feasible to consider EOGRTS as the crucial decision point and critical information requirement in order to decide on ED modality, if this is not a mandatory test in the regulatory requirements. We suggest to redefine critical decision points to align with regulatory requirements and amend conclusions that can be drawn accordingly. We foresee that this would at least

include another option, besides “ED criteria are met/are not met”, namely: “an ED modality based on EATS parameters for mammals cannot be established for this substance”.

25. **Section 3.4.1. Page 28. lines 413-428. Scenarios 1a/1b imply a strong bias towards demonstration of false positives.** The guidance describes that even if adversity based on EATS-mediated parameters is established, a full MoA has to be documented. This is too heavy a requirement and goes beyond the criteria as described in the regulations. Moreover, it contradicts the precautionary principle as it is described in the regulations. In contrast, if no EATS-mediated parameters can be established, this is sufficient to conclude that a substance is not an ED (scenario 1a). We suggest to rephrase the conclusion as follows: “In this case, an ED modality based on EATS parameters for mammals cannot be established for this substance”.

26. **Section 3.4.1. Page 28. Lines 460-489.** According to **scenarios 2a (ii and iii)**, if EATS-mediated parameters are not sufficiently investigated, level 2 and 3 tests are sufficient to conclude on ED modality. In other words, a negative uterotrophic/Hershberger assay can be sufficient to conclude that a substance is not an ED. While these assays can aid in the identification of an ED that act via the ER or AR, these assays are absolutely not sufficient to decide whether or not a substance is an ED. This also acknowledged in the guidance documents in line 1275 (page 54): level 3 assays are incapable of revealing the full spectrum of possible ED effects. Therefore, we strongly suggest to revise conclusion of scenarios 2a. We suggest the following rephrasing “In this case, it is not possible to conclude on an ED modality based on E and A parameters for mammals and more information should be provided”.

27. **Section 3.5.1. Page 32. Line 566-575.** It is unreasonable to assume only one, linear MOA will exist for an ED. Disruption of e.g. estrogen signaling will lead to multiple adverse effects, also depending on the timing of exposure. Also, multiple MoAs may contribute to a single adverse effect (e.g. infertility). It should at least be acknowledged that biology is not linear. Also, starting with the MoA for which the most convincing evidence is available (line 570) is skewed, as this might be not the most relevant MoA. This paragraph needs to be rephrased to better reflect our current understanding of biology or –alternatively- be removed.

28. **Paragraph 3.5.2. Page 33, line 611.** This is too much of a requirement. This type and level of information is not provided with standard information requirements, nor with existing TGs. It is not clear from the guidance document how the absence of these data will impact ED identification. We would welcome clarification from the drafting group on this point as well as concrete suggestions on which information sources and/or experimental tests are expected to provide information about dose concordance, temporal concordance and essentiality, as the guidance describes that these three factors should be assessed to determine biological plausibility.

29. **Section 3.5.2.2 Page 36. Lines 703**. **Dose-concordance**: ED identification it is not a risk assessment, but a hazard assessment. Now, the guidance places a strong focus on dose-response relationships. Also, this guidance leaves a lot of room for testing at low doses (because earlier KEs need to proceed adverse effects, at lower doses) and consequently finding no effects. Effect doses are strongly dependent on the sensitivity of an assay and the endpoints that are measured. Therefore, assessing dose concordance based on different (types of) assays is scientifically flawed. We suggest this part should be deleted.

30. **Section 3.5.2.2 Line 711. Page36**: We highly question whether temporal concordance can be established based on regulatory required tests. Moreover, MIEs and KEs are often studied in vitro, while adverse effects are studied in vivo. Temporal concordance cannot be established from in vitro studies. Therefore, assessing temporal concordance based on different (types of) assays is scientifically flawed. We suggest this part should be deleted.

31. **Section 3.5.2.3 Page 37. Line 733**. In practice, essentiality will always be scored low, as it will never be determined the way it is now phrased. Only in vitro antagonism (at receptor level) might be assessed, but stop dosing (stop-recovery studies) and use of knock-out animals is not common practice in any TG. We suggest to delete this part (lines 733-746) as well as lines 825-929.

32. **Section 3.6. Page 42, Line 878**. “In all other scenarios, the conclusion on the ED properties of a substance should be drawn on the basis of the MoA analysis and the biological plausibility of the link between the adverse effects and the endocrine activity”. Please remove “the MoA analysis” from this sentence. Indeed, the criteria state that the adverse effect should be the consequence of an endocrine mode of action, but this is not the same as what is now outlined in the guidance document, which is that a MoA should be provided. Therefore, the level of evidence that is requested in the guidance document seems to go beyond what is requested in the criteria. We acknowledge that a MoA description can aid to demonstrate biological plausibility (i.e. can a substance lead to an adverse effect as a result of the endocrine activity of a substance), but this should clearly NOT be the main focus of the guidance.

33. **Section 3.6, page 41. Line 921**. The entire guidance is directed towards EATS-mediated ED modality in mammals for which a heavy burden of proof to demonstrate a MoA is required. This is not in line with the ED criteria (line 866). It should be acknowledged here that this is a huge limitation of the guidance. Considering that current TGs mainly focus on EATS, pursuing a non-EATS modality would most likely mean doing scientific research and using non-protocol (OECD) test guidelines. Following paragraph/line 913-918, the guidance does not provide guidance on how to investigate a non-EATS mediated endocrine MoA, as the focus of the guidance is on existing TGs to assess EATS-mediated parameters in mammals. Consequently, any non-EATS mediated endpoint that will be found will lead to the conclusion "biological plausibility is uncertain". The guidance document should clearly acknowledge this narrow, "EATS-mediated for mammals" focus. Also, it should be outlined in this section how other parameters beyond EATS and ED-mediated adverse effects (that are currently not covered in existing TGs) will be included in the guidance document for ED identification in the future, as the scientific knowledge will progress and testing guidelines will be amended/developed in the near future.

34. **Chapter 4 page 44. Line 930.** In chapter 4, it should be stressed that the provided suggestions for test methods is highly amendable. Existing TGs are updated and amended regularly and new test guidelines are continuously being developed. Moreover, there should be a placeholder to expand the guidance document in the future in order to include novel scientific insights into non-EATS parameters and ED-mediated adverse health effects that are currently not covered by existing TGs.

35. **Section 4. Page 46. Line 959.** Please add "including cohorts 2 (DNT) and 3 (DIT)".

36. **Section 4.3.1.2. page 61 Line 1528**. "The rationale for omission of these cohorts should be given". This should be phrased more strongly, for example "Cohorts 2 and 3 should be included by default, so any deviation from the TG by omission of these cohorts is unacceptable, unless strong arguments can be given."

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