

European Food Safety Agency (EFSA)
Scientific Committee
Via Carlo Magno, 1A,
43126 Parma PR, Italy

02 Feb. 2021

RE: Public consultation on the draft EFSA Scientific Committee Opinion on biological plausibility of non-monotonic dose responses and their impact on the risk assessment

Dear Members of the EFSA Scientific Committee,

The Endocrine Society takes this opportunity to provide detailed comments addressing important issues regarding the draft EFSA Scientific Committee Opinion on biological plausibility of non-monotonic dose responses and their impact on risk assessment. In its current construction, the document is an inaccurate assessment of non-monotonic dose responses (NMDRs); without substantial revision, adoption of the opinion will result in the use of restrictive criteria that will limit the ability of regulatory agencies to make health protective decisions. We hope that these comments will help EFSA re-evaluate the scientific basis of the opinion's conclusions. Additional improvements would be possible through collaboration with other stakeholders, including endocrine scientists, to ensure that the final draft accurately reflects the state of the science of NMDRs and their relevance to regulatory assessments, including for endocrine-disrupting chemicals (EDCs).

In the appendix to this letter, we provide line-by-line comments as submitted to EFSA on the public consultation website. However, we wish to highlight several foundational issues that are relevant to the entire document and provide justification for a significant revision of the opinion.

- 1) The opinion fails to acknowledge that NMDRs are well-defined mathematically, have been demonstrated to occur, and are well understood based on basic research of endocrine systems and hormone biology. NMDRs are in fact common and expected features of chemical interference with hormonal systems because the putative causes are involved in multilevel complex regulatory processes. Moreover, NMDRs can and should be assessed statistically.
- 2) The notion of biological plausibility is central to the report; however, plausibility is relative to a body of knowledge, both empirical and theoretical. Without making explicit which body of knowledge the authors refer to, plausibility remains a very vague and subjective notion. It should stand to reason that we can only assess the plausibility of NMDRs for EDCs with knowledge from the discipline of endocrinology. This knowledge pertains both to the general understanding of endocrine systems, and of course, the responses to xenobiotic exposures in specific experiments.
- 3) Despite acknowledging that NMDRs are relevant to efforts for regulating EDCs, the opinion fails to consider and incorporate scientific principles of endocrinology (Zoeller et al., 2012,



Vandenberg et al., 2012) that explain the fundamental biology of endocrine systems and how interference with these systems can produce NMDRs. Participation by endocrine scientists throughout the drafting process is necessary to ensure that these well-established principles are not overlooked in the opinion.

- 4) As described in Montévil et al., (2020) and elsewhere, and consistent with the precautionary principle and a public-health focused approach to chemical regulation, it is unreasonable to insist that NMDRs require a more detailed mechanistic understanding when compared with monotonic dose responses (MDRs) for chemical assessments. Requiring such information for one response and not the other would indicate bias by the agency; an NMDR supported by appropriate statistical analysis is an empirical observation that requires no more information for application to risk assessments or other chemical evaluations.
- 5) The process and approach described in the introduction should be subjected to public and scientific scrutiny in the context of this consultation. The text does not reflect the latest scientific consensus and scientific terms are conflated and inadequately defined (for examples see our specific comments). As such it is not an appropriate foundation for the rest of the document. EFSA should open the entire document for additional public consultation and expert review.
- 6) The extensive use of subjective judgment in the opinion is troubling and lacks transparency. Some assertions in the opinion are made without documentation, explanation, or citation. There is use of biased and negative language to describe NMDR even where they are demonstrated statistically, are replicable, and of relevance to public health. At a minimum, a more transparent framework and description of how the authors arrived at opinions is necessary.
- 7) The search strategy used to identify scientific papers is not comprehensive, limiting the breadth of available science used to arrive at conclusions. Furthermore, the criteria used to select papers for subsequent analysis is also subjective and prone to bias.

By addressing the issues above in the final opinion, EFSA and other scientific agencies will be better positioned to address NMDRs in the course of risk assessments for chemicals that may interfere with endocrine systems and hormone biology. Thank you for considering our comments, if we can be of further assistance, please contact Joseph Laakso, PhD, Director of Science Policy at jlaakso@endocrine.org.

Sincerely,

Barbara Demeneix, PhD, DSc
Chair, Endocrine Society EDC Advisory Group



Appendix: Line-by-line comments submitted via the EFSA Website

Comments on the Introduction:

- Lines 32-40: A more careful review of Vandenberg et al., (2012) [beyond its inclusion in the reference to Beausoleil et al., (2016)] is required. For instance, a mathematical definition of non-monotonicity is included in Vandenberg et al. We emphasize that low-dose effects and NMDR are conceptually different phenomena; these are clearly distinguished in Vandenberg et al., (2012) and should not be conflated in the opinion. Similarly, non-linearity should not be used synonymously with non-monotonicity; for example, a sigmoidal curve is not a linear curve, but it is not non-monotonic. The authors note that Hill et al., (2018) argues for the existence of NMDRs occurring in different parts of the dose response curve, but then fail to acknowledge that the occurrence of responses in different parts of the curve would have different impacts on risk assessment. Furthermore, Hill et al., (2018) identifies 6 NMDRs, and only 2 of them were addressed in this report.
- Lines 47-51: Hormesis is an entirely different concept than NMDR. Hormesis implies beneficial effects, e.g., exposure to a mild stressor conferring resistance to subsequent, harmful conditions of increased stress. Yet, while hormesis is normally considered a beneficial or adaptive response, it is still a non-monotonic response. NMDR must be mathematically defined independent of whether the effect is adverse or not. This is also explained in detail in Vandenberg et al., (2012).
- Lines 51-53: We recommend clarifying the description of Conolly and Lutz (2004) to reflect that they used four examples to demonstrate that competing monotonic curves influencing the same endpoint is one potential mechanistic explanation for an observed NMDR. This is a well described phenomenon when the same chemical or even pharmaceutical has effects on more than one molecular pathway. This is especially important for chemicals which have not been selected for specificity and almost always impact multiple receptor-mediated molecular mechanisms. Importantly, Conolly & Lutz indicate that other mechanisms for NMDRs are also plausible. They state: “Numerous additional mechanisms [for NMDRs] can be put forward, e.g., overshooting homeostatic feedback control or shifts in immune responses, to name just two. Some may simply be derived from superimposition of counteracting monotonic dose responses (Examples #1 and 4), others may be more complex, involving modulation of the activity of endogenous factors (Example #2) or of the background DNA damage (Example #3).”
- Lines 54-62: It is also important in this section to describe the conclusions of a consensus meeting in Berlin from 2012 involving EFSA and other stakeholders which broadly agreed that in the context of risk/safety assessments for food, consumer products and environmental exposures, endocrine disruptors may cause adverse effects at levels relevant to human or environmental exposures; these effects may, in some cases, be represented by non-monotonic dose response curves; and these curves are common enough to warrant formal consideration in risk/safety assessments. This meeting is described in Beausoleil et al., (2013) and Munn and Heindel (2013). Heindel and Munn report that the participants in the Berlin meeting



overwhelmingly agreed (90% of participants) that NMDRs and “low dose effects” are distinct concepts, and that NMDRs exist in some portion of the dose response range.

- Line 75-76: We agree that having at least 5 dose groups is appropriate and necessary for mathematically evaluating a NMDR, but in some sections of the report the committee has required 6 groups (5 treated groups + the negative control) which is not justified.
- Line 84: Statistical treatment is necessary to make this determination. Also note typographical error (monotone vs. monotonic).
- Line 87: Please explain the rationale for the 5% cutoff value. Application of arbitrary cut-offs may limit sensitivity to detect effects and should be statistically determined from study/experimental data. We are concerned that this cutoff would eliminate significant endocrine effects. For example, a change of receptor binding in the 1-3% range would be expected to induce profound biological outcomes. See discussion in Vandenberg et al. (2012).
- Line 88: Please explain how this checkpoint can be fulfilled objectively.
- Line 90: Please clarify what is meant by “2 directions”. Does this refer to multi-phasic rather than bi-phasic curves?
- Line 93-95: We do not agree that visual inspection is sufficient for evaluating the checkpoints according to the methodology presented. This is particularly concerning given that so few studies cleared all of the checkpoints. A more detailed statistical analysis should be used to evaluate whether effects are significant and/or indicative of a NMDR.
- Line 146-147: Please clarify how many publications were represented in the final datasets.
- Lines 152-156: Please clarify the necessity of establishing biological plausibility, in particular why biological plausibility must be established for NMDR as opposed to a linear dose-response when the Bradford-Hill criteria already allow for the possibility of more complex dose-response functions. We note that plausibility changes as scientific advances generate new knowledge of biological systems. For example, mammalian cloning only became possible after scientific advances such as transplantation of frog intestinal cell nuclei into enucleated frog oocytes was shown to produce viable frogs. Demonstration of a non-random phenomenon via statistics should be a valid criterion for establishing plausibility since the methodology to do this for NMDRs is available and does not require further scientific advances.
- Lines 159-162: We maintain that the presence of NMDRs challenges assumptions built into risk assessment approaches, for example the validity of extrapolating information from high dose ranges to assume safety at low doses. We find that the risk assessment process typically relies on the identification of a single key study for the evaluation of hazard and the identification of points of departure (NOAEL or BMD). Yet, we also note that the studies used to identify



NOAEL or BMD levels rarely have sufficient dose groups to detect NMDRs. This is insufficiently addressed by the committee.

- Lines 174-175: Please note that the Endocrine Society is not an academic institution, but rather an international professional scientific and medical specialty society. We welcome the opportunity for international cooperation in evaluating and applying NMDR to regulatory policies; cooperative activities should include the involvement of expert endocrine scientists and members of the Endocrine Society at early stages in the conceptualization and drafting of documents such as this draft opinion given the principal focus identified earlier in the paragraph regarding the assessment of endocrine-disrupting chemicals.
- Lines 216-220: The authors should note that OECD test guidelines, which do not require sufficient dose groups, should be revised to encourage additional dose groups so that more endpoints can be evaluated for NMDR. Furthermore, because NMDR are well-documented in academic studies (as reviewed in numerous Endocrine Society scientific statements – see Zoeller et al. (2012) and Gore et al. (2015)), evaluations should begin with the expectation that a NMDR could be detected and is biologically relevant.
- Lines 226 – 235: The authors should note the similarity of hormones to essential nutrients as described i.e., for many outcomes, there are health effects associated with both deficiency and excess. In the context of regulatory toxicology, the point that should be emphasized is that chemicals that affect the uptake or metabolism of nutrients and hormones can create NMDR. These effects are not well captured by current risk assessment approaches.

Section 2: Data and Methodologies

General Comments

The search terms are too restrictive and will miss many important publications that would be captured using terms such as "biphasic", "U-shape", "inverted U" and "hormesis". Search terms encompassing endocrinology should also be used e.g., "hormone* OR endocrine* OR toxic*". Adding these terms expands the number of publications considerably and would add important research involving chemical interference with endocrine systems and NMDR.

Section 2.1: Data

- Line 258-259: If the search strategy is intended to identify chemicals such as BPA and phthalates for use in the case studies, simple improvements in search terms would identify far more papers for these chemicals. For example, the search ((monotonic OR nonmonotonic OR non-monotonic OR biphasic OR U-shaped OR hormesis OR hormetic OR "inverted U") AND (BPA OR bisphenol A OR bisphenol-A) AND dose) generates 134 results in PubMed. A similar search for specific phthalates yields 34 results.

Section 2.2: Methodologies



- Lines 284-285: We are concerned about the use of expert judgement in the opinion to evaluate NMDRs. In particular, because of the selection of chemicals that are known to interfere with endocrine systems, endocrine scientists must be included as experts in the assessment of these chemicals for health effects.
- Lines 286-287: We caution against a reliance on AOPs, which are frameworks for pathways and are inadequate for describing complex biological interactions. Please also describe how specific AOPs were selected for the outcomes described and if they were approved by OECD.
- Line 289: We recommend that the authors describe and defend their choice of whether outcomes are considered adverse or not.

Section 3:

General Comments

The authors characterize biological plausibility only for well-described physiological phenomena where the detailed mechanism of action is known. However, if the mechanism of action has not been yet completely characterized or is not completely understood then they conclude that the NMDR is not plausible. This is an incorrect application of plausibility for establishing the existence of an observed biological phenomenon based on well-established knowledge of hormonal systems. As described in Montévil et al., (2020) and elsewhere, NMDRs are common and expected features of chemical interference with hormonal systems because the putative causes are involved in multilevel complex regulatory processes due to the evolutionary history of hormones and their functions. We are unconvinced that NMDRs require a more detailed mechanistic understanding when compared with MDRs; regulating EDCs based on observed NMDRs is consistent with endocrine science, as well as the precautionary principle and a public-health focused approach to chemical regulation.

Section 3.1: In vivo studies with datasets fulfilling five or six checkpoints

- Line 312: Biological plausibility is not an ‘intrinsic’ property. Furthermore, plausibility is relative to a body of knowledge, both empirical and theoretical. Without making explicit which body of knowledge the authors refer to, plausibility remains a very vague and subjective notion. We suspect that the authors mean ‘well-understood’ or ‘obvious’.
- Lines 317-320: We caution that the interpretation of a beneficial or adverse effect does not suggest a fundamentally different or separate mechanism for effects seen at different dose ranges.
- Lines 335-341: There are at least 7 well-understood mechanisms for NMDR reviewed in Vandenberg et al., (2012). These include cytotoxicity, cell- and tissue-specific receptors and cofactors, receptor selectivity, receptor down-regulation and desensitization, receptor competition, and endocrine negative feedback loops.



- Lines 377-379: This is an insufficient explanation regarding the use of expert judgement and rationale for the removal of studies from consideration. A more transparent process involving endocrine expertise is required.

Section 3.2: Other studies

- Lines 411-414: The authors should clarify which of the checkpoints for BPA were not fulfilled, this information is not provided in the references cited.
- Lines 430-431: The authors should avoid subjective terms like ‘modest’; the effect observed in the referenced paper was statistically significant with similar dose-responses observed for multiple endpoints. The conclusion that an NMDR “seems unlikely” is subjective and not justified by the statistically significant effects seen in the referenced papers. Furthermore, studies showing an NMDR should not be discredited by comparison with other studies that may have used different rodent strains, species, dosing regimens, and dosing periods; variation across these variables is expected.
- Lines 432-435: While a definitive mathematical evaluation of an NMDR in this instance may not be possible, Taylor et al., (2018) does in fact demonstrate a significant effect at the lower dose range tested that was not apparent at higher doses or in the unexposed group. This information supports the plausibility of an NMDR and should not be excluded from analysis or consideration.
- Line 438: The authors claim that they conducted their own statistical evaluation but do not show the evaluation nor cite a reference. This claim should be removed without supporting evidence, and the conclusion of the paragraph revised accordingly.
- Lines 441-447: As above, we stress that the ‘modest’ effect sizes are important and significant when appropriate statistical treatment is applied. The effects seen, including across animals in different age groups, are highly relevant in the context of susceptibility to disease and other health effects, and therefore relevant for risk assessment. More overt features and effects would be incompatible with life. Furthermore, the statement in line 445 that effects must be coincident with changes in other related biomarkers seems subjective and arbitrary. The statement in line 441 and thereafter should be revised to acknowledge that the effects found were statistically significant and replicable.
- Lines 471-472: It is important to note that the intermediate outcome of lowered testosterone levels is clinically relevant. The age at which testosterone levels are impacted is also important. If serum testosterone levels are disrupted during sensitive developmental windows, endocrinologists would consider this outcome adverse due to various impacts including the most common male birth defects (cryptorchidism and hypospadias).

Section 3.3: Impact of the observed NMDR on the risk assessment process



- Lines 497-500: The conclusion drawn in the opinion is not concordant with Hill et al., (2018), who conclude that effects seen are adverse. To provide transparency and replicability, the authors of this report should define or specify the outcomes that are considered adverse.
- Lines 502-505: We do not agree with this statement. The triggering of an adaptive or homeostatic response during early or intermediate effects due to EDCs is not always benign or beneficial, and there is no evidence provided to support the example. Indeed, it may be impossible to determine if such a change is adaptive or maladaptive, making this statement subjective. For example, changes in a fetus exposed to famine are "adaptive" for an environment of scarcity but become maladaptive for an environment offering access to excess food. This feature is well documented for populations exposed to famine in the course of fetal development during periods of war that were then exposed to plentiful environments later in life leading to cardiovascular disease and early death. See Bateson, P. (2007).

Conclusions

- Lines 571-573: We do not agree that a complete understanding of the mechanism of action is required for establishing biological plausibility for NMDRs. There is more than sufficient understanding of hormonal systems and chemical interference with these systems to conclude that NMDRs are not only biologically plausible but have been demonstrated and should be considered and acted upon when observed in the context of chemical hazard assessment. It is not appropriate to dismiss statistically significant NMDRs.
- Line 592: The authors' conclusion for BPA is not supported by the information in the table which references several studies where an NMDR was observed. We are concerned that these observed NMDR were discounted by the authors' opinions based on subjective judgment, rather than by the use of reproducible, transparent and consistent methods for evaluating NMDRs.

Annex I. Assessment of non-monotonicity claims for BPA

- Lines 898 – 907: This statement incorrectly interprets the experiments described in Montévil et al., (2020). The results were in fact replicated in two independent animal tests and in their publication the authors describe the biological processes that could lead to the observed results. We again recommend that the report's authors avoid using subjective terms (e.g., rather unconventional) when describing a statistically significant and replicable result.
- Line 858-860: The studies referenced here do demonstrate with certainty that lower doses have different effects than higher doses. This should be an important consideration in the context of biological plausibility for NMDR. EFSA must not exclude these studies from consideration.
- Line 927: The CLARITY-BPA study was not initiated due to a lack of consistency among studies, it was initiated to compare the efficacy of the test guidelines, and the endpoints evaluated in those test guidelines, to characterize the effects of exposure to BPA. Further, it was



specifically designed to compare the results in non-guideline endpoints with those traditional measures of toxicity included in the guideline-compliant chronic toxicity study.

Annex II. Assessment of non-monotonicity claims for phthalates

- Lines 1117-1119: As we discussed previously in lines [471-472], changes in serum testosterone levels are associated with numerous adverse effects. The NMDR shown in this study is therefore important, even if the observed apical endpoint showed a different dose response. Furthermore, the authors should note that phthalate metabolites can affect more than one endocrine axis; this is particularly true for thyroid hormone. See e.g., O'Connor et al., (2002) where high doses of DBP led to greater reported effects on circulating thyroid hormones relative to decreases in testosterone.
- Line 1126-1127: The interpretation of total T3 in Meeker and Ferguson (2011) should be interpreted as NMDR rather than “flattening out”. Furthermore, this is another example of an effect that, if occurring during pregnancy, would certainly affect the neurodevelopment of offspring (see e.g., Korevaar et al., (2016), in particular fig. 2) which shows a clear NMDR for free T4 levels and IQ and white/grey matter ratios.