TOX/2021/09

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Draft EFSA Scientific Committee Opinion on biological plausibility of non- monotonic dose responses and their impact on the risk assessment.

Background

1. In 2016, EFSA published the results of a contracted-out report on a systematic review of the existing literature where signs of non-monotonic dose responses (NMDRs) had been observed (Beausoleil *et al*., 2016), referred to as "the Report". In the Report the scientific evidence for such NMDRs was assessed. The systematic review, with two experts reviewing each dataset, was performed in line with the EFSA guidance (EFSA, 2010). The Report extracted dose-response datasets from studies having at least 5 dose groups, which were then analysed by the PROAST software package. The strength of the evidence was characterised using visual/statistics-based checkpoints.

2. In the Report, a total of 202 in vivo studies were found, 23 of which were discounted for data limitations, leaving 179 for further analysis. A total of 311 in vitro and 9 epidemiological studies were identified, of which only 13 of the former and none of the latter were analysed further.

3. The Report then applied 6 criteria to the remaining studies in order to sift out those whose data exhibited a non-monotonic pattern for visual or statistical reasons, in other words, from visual inspection of how well the published curve fits the data points and from applying probabilistic curve-fitting to produce a curve-of-best-fit to the data set:

i. Could the apparent NMDR be explained by random fluctuations around a horizontal dose- response (i.e. no effect at all)?
ii. Could the apparent NMDR be explained by random fluctuations around a monotonic dose- response (MDR)?
iii. Could the apparent NMDR be explained by one single potential outlying dose group?
iv. Was the effect size in one of the directions of the NMDR smaller than 5 %?
v. Was the steepness of the dose-response curve outside the range of biologically plausible/realistic dose-response shapes?
vi. Did the apparent NMDR consist of more (or less) than two directions?

(NB. This last criterion presumably rules out curves that just change slope without changing the sign (+/-) of the slope, for instance when a ligand binds to two receptors with different affinities.)

4. Using this approach, 10 of the 179 studies fulfilled the sift entirely by giving a "no" to all 6 questions (approximately 6%) and 36 of the 179 fulfilled any 5 of the 6 criteria.

5. The EFSA Scientific Committee (SC) was asked to prepare a scientific opinion on the biological relevance, if any, of the apparent non-monotonic dose responses identified in the Report and to address the possible consequences for the human health risk assessments conducted by EFSA. Specifically, the SC was requested:

a) To assess the biological relevance of the non-monotonic dose responses identified in *vivo* in the Report and the follow up probabilistic assessment (Chevillotte et al. 2017a,b), based on visual/statistics/probabilistic considerations.

b) To evaluate the biologically plausible non-monotonic dose-responses and their potential link with adverse effects, considering if the response induction/increase and response inhibition/decrease should be associated to the same or to different adverse outcomes.

c) To assess the impact of any biologically relevant endpoint showing a non-monotonic dose response *in vivo* on EFSA risk assessment outcomes.

d) If risk assessment impacts are identified, to recommend further actions within EFSA priorities, as well as priorities for international cooperation, to improve future risk assessments.

Due to time and resource limitations, the SC used information from the OpenFoodTox database, other EFSA assessments, and the expertise available at the SC and EFSA Panels and Units.

6. The terms of reference of the current opinion specified that it should focus on the NMDR data identified in The Report (Beausoleil et al., 2016.) and the follow up probabilistic assessment (Chevillotte et al. 2017a, b), both of which were primarily focused on statistical considerations for identifying non-monotonicity. Since most toxicological studies use few dose groups, statistical evaluations of non-monotonicity are difficult and subject to random fluctuation. The SC pointed out that statistical considerations cannot address biological plausibility.

7. The SC highlighted special cases such as that for vitamins and minerals where a U-shaped curve results from two distinct but overlapping biological processes, and hormesis, which may involve an adaptive or over-compensatory

response to a chemical stressor leading to an apparent beneficial effect at low doses. Neither of these effects was within the SC's remit and therefore were not addressed in the Opinion.

8. The SC compared the consistency between methods developed by (Beausoleil et al., (2016) and Chevillotte et al. (2017a, b) with the results from the visual/statistical analysis of datasets judged to show potential NMDR (≥5 checkpoints) by the Report.

9. The biological relevance of potential NMDRs identified was assessed by expert judgment, analysing each selected publication. The systematic approach developed considered three key elements:

a) the role of the measured effect in an Adverse Outcome Pathway (AOP), distinguishing between early event, intermediate events and apical effects;

b) the biological plausibility for a non-monotonic dose response, considering the measured effect and information on the mechanistic pathway when available; and

c) the role in adversity for the observed NMDR, considering the EFSA guidance on the selection of Reference Points (RP) for establishing Health-Based Guidance Values.

10. All 36 studies fulfilling at least 5 of the 6 checkpoints in the Report were included in the assessment.

11. In addition, a search for recent scientific literature on the topic was conducted. This was not a new systematic review, but a targeted literature search for gathering additional relevant peer-reviewed publications between 2017 and October 2019, including references and citations from the retrieved articles. Of 19 additional studies found, 6 on BPA and 6 on phthalates were considered relevant. The other 7 were in mixtures and chemicals outside EFSA's remit and so were omitted from this assessment.

The SC assessment

12. The assessment was divided into two sections. The first covered the *in vivo* studies included in the Report and the datasets that fulfilled five or six of the visual/ statistical checkpoints. These data were tabulated.

13. The first table covered studies in the Report that met 5 or 6 of the criteria and were deemed to have well defined biological explanations. These were:

- Resveratrol on indomethicin-induced gastric ulcer;
- Rosmarinic acid on stress-induced freezing response in mice;

- TanshinoneIIA on electrically induced convulsions in mice
- Caffeine on locomotor activity in mice
- Ethanol and acetaldehyde on locomotor activity in rats
- Two bioflavonoids on serum PGE2 levels in rats

14. The second table covered studies in the Report that met 5 or 6 of the criteria and were deemed not to have well defined biological explanations. These were:

- Hepatic markers for HCH exposure
- Acute methylmercury effects
- DDT on DNA methylation
- Acetonitrile on brain AChE
- Lead acetate on body weight and blood pressure
- Triclosan on serum T3

15. The second section discussed other potentially relevant studies from other EFSA activities not covered in the Report, and summarised evaluations done for tropane alkaloids (identified from an Opinion of the EFSA Scientific Panel on Contaminants in the Food Chain (EFSA CONTAM, 2013), as an example of a biologically relevant NMDR), BPA and phthalates, including that for aromatase inhibition by DEHP.

16. The SC presented two case studies in its Annexes, one on BPA and the other on phthalates, where data in the literature had suggested NMDRs, as stated in the Report.

BPA

17. For BPA, the purported effects were on pERK (extracellular signal regulated kinase) signalling in cerebellar cortex, semen quality and renal and gonadal fat pad lipid content.

18. The relevance to risk assessment of the pERK findings, in the absence other related functional measures was questioned. The examined studies on semen quality appeared to give at best modest and often contradictory results. For the fat pads the small number of groups in the examined studies meant that it was not possible to state whether the changes in adiposity followed a NMDR or not.

19. Other reported possible NMDRs, such as measures of fetal urogenital sinus (Uchtmann et al., 2020), mammary gland response (Montevil et al., 2020), percent basophils and modest changes in changes in % basophil and serum bile acid concentrations were deemed to be in need of replication before they could be evaluated further.

Phthalates

20. One effect that is studied in depth that appears to exhibit a NMDR is the effect of phthalates on testosterone synthesis. A number of observations suggest that low-dose phthalates lead to an increase in testosterone levels in male rats due to inhibition of aromatase activity, followed at higher doses by a fall in testosterone levels. The Committee concluded from the observed NMDR was caused by two different modes of action at the low and the high dose levels, that for risk assessment the effects occurring in the lower dose range would be the critical one. From this they decided that in general terms:

- In assessing dose-response relationships for non-monotonicity, the checkpoint approach may yield a different result than those obtained through probabilistic (statistical) methodology;

- Using different statistical approaches may result in diverging conclusions when used individually;

- Apparent NMDR have been observed for early (molecular) or intermediate events, but also for some apical effects relevant for the risk assessment;

- Understanding the underlying mechanism(s) is necessary to assess the biological plausibility of an observed NMDR and to consider the consequences for risk assessment;

- An apical NMDR may arise from two or more modes of action, each with a monotonic dose response. If an adverse effect is observed at lower doses, then this would be used to derive the RP for risk assessment. In the case of nutrients with independent dose-response curves for deficiency and toxicity, the adverse effects on both sides are generally different;

- If an NMDR is observed for an early or intermediate event, leading to an apical effect, the biological relevance of the event should be shown. Molecular or intermediate events leading to effects in opposite directions may be linked to different adverse apical effects at different exposure ranges and not showing an NMDR.

Summary of biological plausibility approach

21. The approach was applied to two case studies: Bisphenol A (BPA) and Phthalates. No indications of NMDR were detected for BPA, while for the phthalate DEHP, indications for a biologically plausible NMDR were observed for an intermediate effect, testosterone levels, possibly linked to the feedback control mechanism. It was recommended that the impact of this NMDR on the risk assessment of DEHP should be further investigated.

Impact on the risk assessment process.

22. The SC stated that NMDRs would only have an effect on the hazard characterisation, i.e. derivation of a health-based guidance value for a given chemical, if the effect was observed at the low dose end of the dose response curve since the effect occurring at the lowest applied dose was the critical effect which would define the reference point (RP) upon which the HBGV would be based. Non-monotonicity higher up the dose range may have different causes, such as saturation of detoxification pathways or toxicity by other mechanisms.

23. Moreover, some literature observations of *in vivo* NMDRs relate to intermediate effects in a response to exposure. If these do not then go on to have an effect on the apical outcome, then these will not affect hazard characterisation.

24. The identification of valid low dose NMDRs depends upon whether the study design includes sufficient dose spacing over the region of the curve where the critical effect is observed to take place.

Recommendations

25. The SC recommended, for addressing an apparent NMDR for risk assessment, that the following procedure should be followed:

26. If non-monotonicity is found at the upper end of the dose-response curve, then the current approach for determining a RP and establishing an HBGV should be followed.

27. If monotonicity is found at the at the lower end of the curve, further considerations need to be taken into account as follows:

- Is the effect observed an apical effect and is it supported by further experimental work? If not, further investigations are needed. If the observed effect is an early or intermediate effect, consider:

  - The evidence for the effect observed (*in vitro/in vivo*? Other?).

  - The biological relevance of the effects observed. Can a (quantitative) relation between these effects and an adverse outcome (i.e., apical effect) be established? Ideally, could a mechanistic sequence (AOP) be partially or fully established? If so, specific considerations need to be applied and a deviation from the current methodologies for RA as described in EHC 240 (IPCS, 2009) or FOSIE (Barlow et al., 2002) may be needed.

  - If information is lacking on whether an observed effect can lead to an adverse outcome, additional testing may be needed. New Approach

Methodologies (NAMs) could be used here to identify a mechanistic sequence of events.

Discussion of the draft opinion

28. Non-monotonicity is difficult to ascribe because the effect can be achieved by different overlapping mechanisms that may themselves have monotonic dose responses. Moreover, regarding the relevance of an apparent NMDR to risk assessment, the SC point out that firstly, only a NMDR in the low-dose end of a dose-response curve would have consequences for the derivation of a HBGV and secondly, that apical adverse events follow adverse outcome pathways where the apical outcome, upon which a risk assessment would be based, can still have a monotonic dose-response curve, despite apparent non-monotonicity in intermediate events.

29. The paper is difficult to follow with some repetition but makes the point that assessing the biological plausibility of any purported NMDR is necessary before its impact upon risk assessment for the chemical involved, and this cannot be accomplished by statistical studies or visual inspection alone. A list of recommendations for risk assessors faced with a NMDR is included.

Questions for the Committee

30. Members are asked for any comments they have on the contents or recommendation of the draft opinion.

Secretariat

January 2020

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Review of the EFSA Scientific Committee Opinion on biological plausibility of non- monotonic dose responses and their impact on the risk assessment.

The link to the draft Scientific Committee opinion is given below.

https://www.efsa.europa.eu/en/consultations/call/public-consultation-draft-efsa-scientific-committee-opinion-0

Secretariat

January 2020

References

Beausoleil C, Beronius A, Bodin L, Bokkers BGH, Boon PE, Burger M, Cao Y, De Wit L, Fischer A, Hanberg A, Leander K, Litens-Karlsson S, Rousselle C, Slob W, Varret C, Wolterink G and Zilliacus J, 2016. Review of non-monotonic dose-responses of substances for human risk assessment. EFSA Supporting Publications, 13:1027E. doi: 10.2903/sp.efsa.2016.EN-1027.

Barlow S, Dybing E, Edler L, Eisenbrand G, Kroes R and Brandt PA, 2002. Food Safety in Europe (FOSIE): Risk Assessment of Chemicals in Food and Diet. Food and Chemical Toxicology, 40

Chevillotte G, Bernard A, Varret C, Ballet P, Bodin L and Roudot AC, 2017a. Probabilistic assessment method of the non-monotonic dose-responses-Part I: Methodological approach. Food Chem Toxicol, 106:376-385. doi: 10.1016/j.fct.2017.05.070

Chevillotte G, Bernard A, Varret C, Ballet P, Bodin L and Roudot AC, 2017b. Probabilistic assessment method of the non-monotonic dose-responses-Part II: Robustness assessment. Food Chem Toxicol, 110:214-228. doi: 10.1016/j.fct.2017.10.030

EFSA (European Food Safety Authority), 2012. EFSA's 17th Scientific Colloquium on low dose response in toxicology and risk assessment. EFSA Supporting Publication 2012; 9(11):EN- 670 353, 64 pp. doi:10.2903/sp.efsa.2012.EN-353 671

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2013. Scientific Opinion on Tropane alkaloids in food and feed. EFSA Journal 2013; 11 (10):3386, 113 pp. doi:10.2903/j.efsa.2013.3386

Hill CE, Myers JP and Vandenberg LN, 2018. Nonmonotonic Dose-Response Curves Occur in Dose Ranges That Are Relevant to Regulatory Decision-Making. Dose-Response, 16. doi: 10.1177/1559325818798282

IPCS (International Programme on Chemical Safety), 2009. Environmental Health Criteria 240, Principles and Methods for the Risk Assessment of Chemicals in Food. World Health Organization.

Montévil M, Acevedo N, Schaeberle CM, Bharadwaj M, Fenton SE and Soto AM, 2020. A Combined Morphometric and Statistical Approach to Assess Nonmonotonicity in the Developing Mammary Gland of Rats in the CLARITY-BPA Study. Environmental Health Perspectives, 128:057001. doi: doi:10.1289/EHP6301

Uchtmann KS, Taylor JA, Timms BG, Stahlhut RW, Ricke EA, Ellersieck MR, vom Saal FS and Ricke WA, 2020. Fetal bisphenol A and ethinylestradiol exposure alters male rat urogenital tract morphology at birth: Confirmation of prior low-dose findings in CLARITY-BPA. Reproductive Toxicology, 91:131-141. doi: https://doi.org/10.1016/j.reprotox.2019.11.007