Questions & Answers

on scientific study 'A combined morphometric and statistical approach to assess non-monotonicity in the developing mammary gland of rats in the CLARITY-BPA study'

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This Questions and Answers document (Q&A) was developed by the Health and Environment Alliance (HEAL). It is based on our reading and analysis of the study and the explanations that it provides on the context of its development, the methodology used and the results obtained.

This Q&A aims at highlighting methodological considerations and findings that appear relevant to us in the context of the analysis of health effects of bisphenol A. It further seeks to help translating some of the complex aspects of this study into accessible language and highlighting their relevance to the current regulatory developments around bisphenol A.

Further questions about the study should be directed to the study authors.

1 Why is this study particularly relevant in comparison to previous studies about the effects of BPA?

The study brings together different types of data analysis in order to assess the effects of BPA on the mammary gland. On the one hand, it uses traditional semi-quantitative methods; and on the other hand, it uses a novel quantitative method that has been developed specifically in the context of this study in order to precisely assess the dose response curves. The development of such measurement methods is one of the objectives of the CLARITY-BPA project.

This statistical method (permutation test) used for the first time in this field of research makes it possible to provide a rigorous analysis of the non-monotonic dose response curve.

- It allows to bring a statistical confirmation of the results obtained through the semi-quantitative approach.
- It uses five BPA measurement doses over a wide dosing range in order to cover a long time of exposure and to properly explore the shape of the dose response curve to BPA for the various endpoints covered in this study.

2 The study is based on animal studies. How useful is the study when considering BPA effects in humans?

Reliance on animal studies is currently necessary to study the health effects of chemicals in-depth, and mammalian models are currently the most developed ones for safety assessment. The rodent mammary gland is considered a sensitive target for endocrine disruption, and the use of the rat model

is considered a better choice than the mouse one regarding mammary cancer. The mouse model is used instead to address effects of perinatal exposure on the foetal and postnatal morphogenesis of the mammary gland. Therefore, this study is particularly relevant in terms of approach and focus when it comes to bringing new insights about the effects of BPA on human health.

The study makes important contributions to existing scientific evidence about the effects of BPA in a number of ways:

- The development of a software tool for computer driven analysis is relevant because the rat mammary gland is more difficult to analyse than that of the mouse. It therefore allows deepening the analysis of the effects of BPA on the rat mammary gland in a way that had not been possible before.
- The study covers critical windows of development, with experiment sets covering periods respectively between gestation and weaning (PND 21), and between gestation until tissue harvesting up to 6 months of age.

The study demonstrates that:

- Developmental exposure to BPA reveals a non-monotonic dose-response curve on mammary gland development at all ages examined, showing a break between the 25ug/kg/day and 250 ug/kg/day doses;
- Perinatal BPA exposure alters mammary gland development at all ages studied, and
- The mammary glands from the dose group terminating at weaning (PND21) provide a very sensitive endpoint to assess developmental toxicity.

3 What is the added-value of the novel quantitative method developed in the context of this study?

This method allows to assess several aspects of mammary gland morphology that are not accessible by manual assessments. This can be because such manual assessments would take too much time, because they are about the gland's 3D properties, or because they require an analysis of the gland as a multi-scale object. The study shows that several aspects of the mammary gland morphology seem to respond to BPA exposure and suggests that these should therefore be further investigated in future studies.

The automatic method is also useful for enhancing the semi-quantitative methods and verifying their findings (not for replacing them). Manual assessments are subject to subconscious biases. With an automatic or semi-automatic method, such biases are far less likely to happen. In this study, such biases were not possible because measurements were done without knowing the treatments, technically referred to as a "double blind study".

4 What is the specific contribution of this study in the scientific understanding of non-monotonic dose-response curves?

Non-monotonic dose responses (NMDR) occur when the slope of the curve relating dose and effect changes sign at some point within the range of the doses examined – a phenomenon of particular relevance in the case of hormones and EDCs. The characterisation of such dose-response curves is

particularly important for risk assessment, because it makes it difficult to predict safe levels of exposure.

Thanks to the use of the novel quantitative method (described in above questions) on a sensitive animal model for a sensitive health endpoint, this study brings out new and precise evidence about the occurrence and characterisation of the non-monotonic dose-response curves for bisphenol A. This response is confirmed by other endpoints in a single, rigorous, statistical analysis.

Not only does the study show the occurrence of low dose effects and non-monotonicity, but it also highlights in an interesting way that non-monotonicity can have different shapes than the inverted "U-shaped" (or quadratic) responses traditionally associated to references to non-monotonicity. Features related to thickness, duct width, fractal dimension in 3D are thoroughly described and of interest.

5 How relevant are the doses considered in the study in comparison to human exposure to BPA?

The doses considered in the study range from 2.5 ug/kg/day to 25000 ug/kg/day, with the lowest dose of 2.5 ug/kg/day being fully consistent with the CLARITY-BPA core study lowest dose.

Worryingly, most of the effects, such as mammary cancer, are already observed at the dose of 2.5 ug/kg/day.

In this regard, the study results reveal both harmful effects at very low doses of BPA exposure and the break of linear dose-response curves between the doses of 25 to 250 ug/kg/day. Overall, these results suggest that regulatory authorities should aim at minimising exposure to BPA. By way of comparison, in 2015 EFSA reduced the tolerable daily intake (TDI) for BPA at the level of 4 ug/kg/day.

Previous studies exploring low dose effects showed adverse effects at 25 ng/kg/day on mice and at 250ng/kg/day on rats.

By way of example:

- Mammary gland development in mice:
  Muñoz-de-Toro M, Markey CM, Wadia PR, et al. Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. Endocrinology. 2005;146(9):4138-4147. doi:10.1210/en.2005-0340

- Effects on reproduction in mice:
  Cabaton, N.J., Wadia, P.R., Rubin, B.S., Zalko, D., Schaeberle, C.M., Askenase, M.H., Gadbois, J.L., Tharp, A.P., Whitt, G.S., Sonnenschein, C., and Soto, A.M. Perinatal exposure to environmentally relevant levels of Bisphenol-A decreases fertility and fecundity in CD-1 mice. Environ. Health Perspect. 2011; 119: 547-552.

  Acevedo N, Rubin BS, Schaeberle CM, Soto AM. Perinatal BPA exposure and reproductive axis function in CD-1 mice. Reprod. Toxicol. 2018 Aug;79:39-46.

- Effects on the metabolome in mice:

  Cabaton NJ, Canlet C, Wadia PR, Tremblay-Franco M, Gautier R, Molina J, Sonnenschein C, Cravedi J-P, Rubin BS, Soto AM, Zalko D: Effects of low doses of bisphenol A on the

metabolome of perinatally exposed CD-1 mice. Environmental Health Perspectives 2013, 121: 586-93

- Metabolic disruption in rats:

  Tremblay-Franco M, Cabaton NJ, Canlet C, et al. Dynamic Metabolic Disruption in Rats Perinatally Exposed to Low Doses of Bisphenol-A. PLoS One. 2015;10(10):e0141698. Published 2015 Oct 30. doi:10.1371/journal.pone.0141698

6 How does this study fit in with the other results under the CLARITY-BPA project?

This study is particularly interesting because it is part of the CLARITY-BPA project. It uses a subset of animals from the CLARITY-BPA core study animals, and generally reflects the initial objectives of the CLARITY-BPA project to allow for a collaboration between standard test methods and academic-based studies in order to analyse the health effects of BPA.

The results obtained and documented about low-dose effects and non-monotonicity are fully consistent with the CLARITY-BPA core study itself, which revealed a significant increase of mammary cancer incidence at the lowest dose of BPA administered (2.5 ug/kg/day).

The study demonstrates a causal link between the doses of BPA administered and the effects observed. Therefore it brings a counterpoint to earlier statements, according to which the low dose effects observed were due to random events.

In this regard, this study provides an important contribution to the CLARITY-BPA project, which is ongoing and of which final results were originally expected for the end of 2019. More information on the CLARITY-BPA project can be found here:
https://ntp.niehs.nih.gov/whatwestudy/topics/bpa/index.html

7 How relevant is this study in the context of recent European regulatory developments?

The publication of this study is relevant in the light of several of the ongoing European regulatory developments:

- The European Food Safety Authority (EFSA) is currently re-assessing the potential hazards of BPA in food and the conclusion of its assessment is expected later in 2020, including based on the outcomes of the CLARITY-BPA study. In 2015, the authority established a tolerable daily intake (TDI) for BPA at the level of 4 ug/kg/day. This new study, part of the CLARITY-BPA study, suggests that effects harmful to human health can happen at much lower doses and that this level should be reviewed.

- At the end of 2019, the European Commission launched a fitness check on endocrine disruptors, including a public consultation (which closed at the end of January 2020). The results of this exercise, expected after the summer, will provide the basis for the revision of the 1999 European EDC strategy, including proposals for addressing identification of EDCs across sectors and how to regulate them. Due to EDC specificities, a long-time demand from scientists and the public health community alike has been to consider that there is no safe dose of exposure for EDCs and as a result to aim at minimising exposure as much as possible (including through a ban in consumer

products). The findings of this study about a well-known endocrine disruptor confirm the relevance of this demand for regulatory purposes.

⑧ How useful is the study for the study of the effects of other bisphenols?

Bisphenols are a large class of chemicals. Because of increasing regulation of BPA, the use of other bisphenols is on the rise. These other bisphenols have been less studied than bisphenol A, although concerns about similar toxicological profiles and properties have been emerging in the last years. Bisphenol S is currently being evaluated under REACH and the proposal to identify bisphenol B as a substance of very high concern due to its endocrine disrupting properties will be discussed in December 2020.

The European Chemicals Agency (ECHA) recently announced its intention to increasingly approach chemical assessment by groups rather than individual substances. This makes sense for bisphenols as well as other groups of chemicals. When indications exist that different substances from the same group might act in similar ways and have the same uses, concerns about the harmful properties of one substance having led to regulation should be used for potential regulation of the entire group.

In this regard, the concerns about low dose effects and non-monotonic dose response curve of BPA highlighted in the new published study might be useful to consider in the light of further research and assessments currently being done on other bisphenols.

(Further reading on the group of bisphenols: Chem Trust, "From BPA to BPZ: a Toxic Soup", March 2018 and "Why a group restriction of the bisphenols is long overdue", 12 May 2020)

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