Appendix to:
The challenge of the application of ‘omics technologies in chemicals risk assessment: background and outlook

Responses from the written inquiry presented in Section 2 of the survey

The following questions were raised in the written inquiry:

1. Has your company already included ‘omics data in any regulatory dossiers/submissions?

2. If so, can you share details on:
   a. The specific type of ‘omics data used;
   b. The technology applied to generate and store data and to analyse and interpret data;
   c. The toxicological endpoint addressed;
   d. How the ‘omics data contributed to hazard categorisation, the determination of NOAEL / NOAEC values, etc.;
   e. The acceptance of such data by the responsible authority /authorities.

3. If you have not yet included ‘omics data in regulatory dossiers, would you be in a position to share with us further details on:
   a. The reasons why you refrained from doing so;
   b. Specific prerequisites that you believe should be met to foster applicability of ‘omics technologies for chemicals risk assessment.

In the following, all responses received are presented (with minor editing from the authors of the present survey). Figures in brackets relate to the respective numberings of the questions listed above.

Respondent A:

(1) Yes.

(2a) Transcriptomics.

(2b) The procedure was basically conducted in accordance with the Affymetrix or Agilent protocol. The obtained image data were quantified using Expression Console (Affymetrix) or Feature Extraction (Agilent). Global normalisation with Expression Console (Affymetrix) or the 75th percentile normalisation (Agilent) was used for per sample normalisation. Comparison analyses were conducted based on the Welch’s t-
test (between control and treatment groups). ANOVA was used if a control group was compared with treatment groups. Gene spring (principal component analysis (PCA) or hierarchical clustering) and Metacore (pathway analysis) were used to interpret the data.

(2c) MoA categorisation for hepato-carcinogenesis.

(2d) Classification based on data similarity by the hierarchical clustering method.

(2e) Under consideration.

Respondent B:

(1) No.

(3a) We do not see that ‘omics data would presently deliver reliable results, based on which we could adequately protect our workers and the environment. Neither do we see that such ‘omics data could be used for the derivation of DNEL or predicted no effect concentration (PNEC) values or for C&L or registration under REACH, above all that ‘omics data would satisfy the standard information requirements of Annex VII of REACH which are presently (before the 2018-timeline to register the so-called ‘phase-in substances’ under REACH) key in our work.

(3b) First, ‘omics technologies must deliver reliable endpoint-relevant results, which can be used for the derivation of DNEL or PNEC values and for C&L according to CLP. After this first criterion is fulfilled, the Commission and the ECHA shall define clear conditions under which the results from ‘omics would be recognised for regulatory purposes under Environment, Health and Safety (EHS) legislation, CLP, and REACH.

Respondent C:

(1) Not in REACH dossiers directly, but it might be that ‘omics were applied to support the occupational exposure levels (OELs) that we use. For benzene, the US Environmental Protection Agency (EPA) relied on genomics data (not developed by industry) in their ‘NexGen’ risk assessment program. Benzene was a pilot substance to demonstrate the future of risk assessment, and not to facilitate a regulatory decision. Our company tried to use this data internally for OEL setting, but the limitation here was that we did not know the MoA, we did not have access to the data or have a clear understanding of how the data were analysed (https://cfpub.epa.gov/ncea/risk/recresult.cfm?deid=286690). There are some ‘omics projects in the pipeline, though: An example is the 'omics study at Concawe
that aims at supporting grouping of petroleum streams and read across within such groups (its pilot study, which also includes methodological details, has been published by Grimm et al. (2016)). This study will, however, not be used directly for MoA arguments in REACH dossiers or for a specific toxicological endpoint.

(3a) We do not use ‘omics data for regulatory purposes, since there is still too much noise in the data and they are too variable: data generation is uncontrolled (timeframe from exposure etc.) and the outcome is too experimental condition-specific to be meaningful. Anyone can publish ‘omics data, and we would have no way of verifying whether the data is accurate or not. Further, it continues to be difficult to correlate ‘omics data to actual adverse effects, let alone to determine human relevance.

(3b) At this moment, ‘omics might be useful to support the POD, but we are hesitant to use it as a POD for the above limitations. In general, the data should be more transparent and accessible. And if it is not already part of the ECETOC workshop Applying ‘omics in chemicals risk assessment, application of assessment factors to ‘omics data should be discussed. This would be whether the full set of assessment factors are required when the start of a chemical risk assessment is based on ‘omics.

**Respondent D:**

(1) We regularly utilise *in vitro* gene expression data for early internal decision making as well as *in vivo* to help guide our testing programmes; i.e. as supporting data for all crop protection products. In the case of industrial chemicals, it is more performed on a case-by-case basis. For crop protection products, we are doing proactive ‘omics evaluation (Next Generation Sequencing (NGS), transcriptomics) as a default. As these molecules move through the pipeline, we will submit the respective data to the regulatory agencies.

(2a) There are only a few situations where we have utilised full ‘omics data via microarray or NGS for a regulatory submission. These have been situations where we have wanted to dig into a very complex MoA.

(2d) In some cases, the authorities have utilised the gene expression data to help support a MoA and determine relevance, or lack thereof, to humans. In other cases, they have driven the NOAEL off of the gene expression data alone! In one case, the authority allowed us to waive the 2-year cancer bioassay based upon MoA and gene expression data.
(2e) Feedback from the regulatory agencies has been excellent (re. crop protection products; and in the USA).

**Respondent E:**

(1) In general, the only truly regulatory submissions would be for pesticide registrations.

(2d) For important commodity chemicals, there have been research studies of using ‘omics approaches for product stewardship and for establishing MoA.