EFSA Scientific Colloquium N°22

'Epigenetics and Risk Assessment: Where do we stand?'

14-15 June 2016 | Valencia, Spain

BRIEFING NOTES FOR DISCUSSION GROUPS

These briefing notes aim to provide participants with the relevant background information so as to be prepared for an interactive exchange of views and expertise during the Scientific Colloquium.

Background

Epigenetic changes are molecular changes mainly in chromatin, such as DNA methylation, histone modifications, that modulate gene expression directly or indirectly through the expression of non-coding RNAs. There is increasing evidence to suggest that individual lifestyles, nutrition and environmental stressors can affect epigenetic processes and as a result, alter phenotypes, longevity, health and disease both within generations (from embryogenesis to adulthood) and in a trans-generational manner. Considerable research in this area has focused on vertebrates (including human), although the number of studies published on invertebrate species and plants is rapidly increasing.

Objectives

This colloquium will bring together experts dealing with epigenetics and human health, animal health and environmental risk assessment of chemicals. There will be an open scientific debate on where we stand regarding knowledge of epigenetics mechanisms and the potential use and integration of epigenetic data in chemical risk assessment.

Participants at the colloquium will critically discuss whether epigenomics data should be integrated into risk assessments, and whether this would improve hazard identification and characterisation, and make a meaningful contribution to the risk assessment process. A further objective aims at addressing molecular mechanisms, methods to investigate epigenetic effects *in vitro* and in *in vivo,* the use of epigenetic biomarkers and identifying existing data gaps and research needs.

Discussion Group 1 | Incorporating epigenetics data in mode of action and adverse outcome pathways frameworks

Introduction

Epigenetic changes are molecular changes in the chromatin that modulate the expression of genes. The changes include DNA methylation, histone modifications and the expression of non-coding RNAs. There is increasing evidence to suggest that life style and environmental stressors can affect epigenetic processes and as a result alter phenotypes, longevity, health and disease both within generations (from embryogenesis to adulthood) and in a trans-generational manner (Kaelin & McKnight, 2013; Lalevee & Feil, 2015; Qureshi & Mehler, 2013).

Mode of action (MOA) and adverse outcome pathways (AOP) are frameworks that provide a basis for identifying sequential chains of causally linked and empirically observable events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect. These frameworks have been developed over the past 10 years as central elements that provide a mechanistic basis to support chemical risk assessment (Edwards et al., 2016; Meek et al., 2014; Simon et al., 2014). While the MOA and AOP frameworks have commonalities and with regards to the identification of measurable key events (biological perturbations) that are necessary for toxicity, a key difference is the inclusion of toxicokinetic processes in the MOA framework whereas the AOP framework was initially thought to encompass chemical-biological interaction.

Recent attempts have been made to use OMICs data, and in particular transcriptomics data, in MOA frameworks through the identification of functional pathways and their incorporation as key events (Moffat et al., 2015). The aim of this discussion group is to critically discuss whether epigenetic events that will have an impact on gene expression can be identified and integrated in mode of action and adverse outcome pathways frameworks as key events. In addition, the discussion group will identify data gaps and research needs.

Discussion points

1. Discuss key epigenetic mechanisms and their potential relevance to key events under MOA/AOP analysis
2. What *in vitro*, *in vivo* assays and non-testing strategies are available to generate relevant data on epigenetic changes for incorporation into MOA analysis and AOP development? Discuss study design (choice of tissues, experimental time points, target organ, cellular model, in silico tool) and biological relevance.
3. How can epigenomics data be integrated with other OMICs data for MOA analysis and/or AOP development?
4. What are the data gaps and research needs?

Background documents

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Discussion Group 2 | The impact of epigenetics on human risk assessment of chemicals

Introduction

Human risk assessment of chemicals is traditionally based on the identification of the hazard posed by the chemical of interest, the characterisation of the hazard through a battery of *in vivo* and *in vitro* experimental tests and the derivation of health-based guidance values that are compared with exposure data. *In vivo* studies used to identify and characterise hazards typically include among others, body weight, organ weight, gross and histopathology, serum chemistry, haematology, reproductive function. Using the NOAELs reported from these *in vivo* studies or following benchmark dose (BMD) modelling of the experimental data, predictive cancer-based and non-cancer-based reference points (RP) (points of departure) are then identified and used to establish appropriate health-based guidance values.

In addition to the biochemical or pathology-based types of experimental data, molecular data derived from OMICS technologies (transcriptomics, proteomics, metabolomics) have also recently received attention for their applicability in hazard characterisation (EFSA, 2014). Indeed, *in vivo* toxicogenomics and toxicoproteomics data have been successfully applied to BMD modelling and predictive cancer-based and non-cancer-based RP identification and have been reported to complement other toxicity data (Moffat et al., 2015; Thomas et al., 2011; Thomas et al., 2012; Webster et al., 2015; Chepelev et al., 2014). Thus, OMICs technologies have the potential to provide important data in support of hazard identification and hazard characterisation.

Epigenetic changes, through DNA methylation, histone modifications and the expression of non-coding RNAs, modulate the expression of genes, and hence contribute not only to cellular mRNA and protein levels but also novel biomarkers. The aim of this discussion group is to critically discuss whether the epigenomics data can be integrated chemical risk assessment by supporting the identification of RP for hazard characterisation. In addition the discussion group will identify data gaps and research needs.

Discussion points

1. Do toxicity studies with their current design allow to measure adverse effects resulting from epigenetic changes? Give examples.
2. Discuss key issues to integrate *in vivo* data reporting epigenetic effects, including non-coding RNA-based biomarkers such as miR-122, in BMD modelling or identification of RP for hazard characterisation?
3. What *in vivo, in vitro* assays and non-testing strategies are available to generate relevant data on epigenetic changes for incorporation into risk assessment frameworks? Discuss study design (choice of tissues, experimental time points, target organ, cellular model, *in silico* tools) and biological relevance.
4. What are the data gaps and research needs?

Background documents

Chepelev, N. L., Meek, M. E., & Yauk, C. L. 2014. Application of benchmark dose modeling to protein expression data in the development and analysis of mode of action/adverse outcome pathways for testicular toxicity. Journal of Applied Toxicology, 34: 1115-1121.

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Discussion Group 3 | Epigenetics in risk assessment of farmed animals for food production: what do we need to consider?

Introduction

Risk assessment of animals used for food production focuses on the identification of potential hazards, hazard characterization and quantitative estimates of risk. Scientific advances in the understanding of biology can improve the hazard identification and characterization processes. Potential new tools need to be assessed for their usefulness, including whether the science is sufficiently advanced for the tool to improve hazard identification and be incorporated into the risk assessment process.

Changes in an animal's epigenome can result in phenotypic changes, without changing the primary sequence of that animal's DNA. An animal's epigenome is dynamic and subject to change over time. It can be influenced by a variety of factors, including: stage of development, nutrition, environment, disease, or social factors. These changes can result in a positive or negative outcome for the animal (e.g. alter normal developmental processes or disease susceptibility). Some of these outcomes can result in alterations of the phenotype that are transient, while others may have permanent impacts on animal health, including disease susceptibility and longevity, from embryogenesis to adulthood and potentially in a trans-generational manner. These phenomena have been studied in farmed animals (Freeney et al. 2014), but only to a relatively limited extent.

Animal breeding is focused on animal improvement and the heritability of certain phenotypes; epigenetic variability can influence these phenotypes, including production traits. Currently breeders employ modern biotechnologies such as genomic selection, semen sorting, in vitro fertilisation, and cloning. Some of these techniques have been evaluated for their effects on the epigenetic status of the adult animals (e.g., for cloning, see US-FDA (2008), EFSA (2008), Japan FSC (2009), for in vitro embryo production (Smith et al., 2015)). In the near future, gene editing and gene drive technologies may be added to the list; these have the potential to more precisely control genomic selection and manipulation, and may allow for epigenomic editing (Qi et al., 2013).

The aim of this discussion group is to critically discuss the current state of the field of epigenetics, including whether any subset of marks and changes to those marks can be causally associated with phenotypic changes and whether sufficient information exists to use these to characterize potential hazards and make inferences regarding animal health or food consumption risks. In addition, the discussion group will identify data needs, data gaps, and potential research needs.

Discussion points

1. What are key epigenetic marks? Are changes in these marks biologically significant? (How is "normal" defined, biologically and statistically?)
2. What are the available methods for assaying epigenetic marks? What would be necessary to validate them?

3. Do changes in epigenetic marks lead to predictable phenotype changes?
4. What are the knowledge gaps and how they can be addressed?

Background documents

EFSA, 2008. Food Safety, Animal Health and Welfare and Environmental Impact of Animals derived from Cloning by Somatic Cell Nucleus Transfer (SCNT) and their Offspring and Products Obtained from those Animals. Chapter 3: Epigenetic and genetic aspects of SCNT. The EFSA Journal (2008) 767, 1-49.

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Japan Food Safety Commission, 2009. Risk assessment report on foods derived from clones cattle and pigs produces by somatic cell nuclear transfer (SCNT) and their offspring (novel foods). Chapter V epigenetics and other genetic properties for SCNET cloned animals.

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Discussion Group 4 | Epigenetics and environmental risk assessment: mechanisms, testing and data gaps

Introduction

In the food safety area, environmental risk assessment of chemicals aims to maintain a healthy environment and conserving biodiversity with regards to regulated compounds used in agriculture such as plant protection products and feed additives as well as environmental contaminants. The risk assessment frameworks require a number of toxicological studies to ensure the safety of those compounds, before they reach the market, including acute and chronic toxicity studies in test species such as fish, earth worms and daphnia. However, standard test species may not always address the toxicological sensitivity of endangered species. For this purpose, EFSA has proposed in its scientific opinion on "coverage of endangered species in environmental risk assessments at EFSA" to address sensitivity for both toxicokinetic (TK) and toxicodynamic (TD) processes with regards to taxa-specific traits, interspecies differences and inter-taxa differences (EFSA, 2016).

In considering toxicological aspects, recent advances in "eco-toxicogenomics" have given the opportunity to integrate the results of omics studies at the gene, protein, metabolome and epigenetic level (i.e., transcriptomics, proteomics, metabolomics, and epigenomics)(Kim et al., 2015). Epigenetic mechanisms typically involve DNA methylation, histone modifications and expression of microRNA and an increasing number of species have been investigated in this regard including algae, plants, daphnia and fish (Mukherjee et al., 2015; Vandegehuchte and Janssen, 2014). Recently, the Adverse Outcome Pathway (AOP) concept has been proposed to guide research aimed at improving both understanding toxicity for chronic endpoints, including delayed toxicity, epigenetic and transgenerational effects and as a tool to increase the ability of the scientific community to predict adverse outcomes (Groh et al., 2015).

The aim of this discussion group is to critically discuss eco-toxicological laboratory studies and their ability to investigate epigenetic effects and mechanisms, epigenetic biomarkers and tools to investigate differences in epigenetic targets and mechanisms. Finally, the discussion group will identify data gaps and research needs.

Discussion points

1. Do eco-toxicological laboratory studies with their current design allow to measure adverse effects resulting from epigenetic changes? Give examples.
2. Discuss key issues to integrate *in vivo* data reporting epigenetic effects in eco-toxicological studies. They may include biomarkers for non-coding RNA, DNA methylation and histone modifications in dose response modelling/ hazard characterisation?
3. Considering species of environmental relevance, what are the tools and data available to investigate differences in epigenetic targets and

mechanisms? Discuss molecular and bioinformatics tools, databases and experimental models.

4. What are the data gaps and research needs?

Background documents

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