COMMITTEES ON CARCINOGENICITY, MUTAGENICITY AND TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC, COM and COT)

Statement from a joint Committee workshop on the use of epigenetics in chemical risk assessment

https://www.gov.uk/government/groups/committee-on-carcinogenicity-of-chemicals-in-food-consumer-products-and-the-environment-coc

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Preamble

The field of epigenetics and the potential role of epigenetic changes in toxicology have been considered previously by COC, COM and COT, and all have recently recommended maintaining a watching brief on developments in their respective Horizon Scanning exercises. To fulfil this brief, a workshop for Members of the three Committees was organised in October 2017 with the aim of considering the overarching question; '*Whether epigenetics should be used in chemical risk assessment'*. Three speakers were invited to give presentations to provide background to the consideration of this question in breakout discussion groups. This statement summarises information from the presentations given at the workshop, the outcomes of the breakout group deliberations and the subsequent discussions and conclusions.

Introduction

1. Epigenetics is defined, in this statement, the study of heritable changes in gene function that occur without a change in the sequence of nuclear DNA and the processes involved in the unfolding development of an organism (https://www.epigenesys.eu/en/learn/glossary/epigenetics). However a number of meanings of epigenetics exist (Greally, 2018). Many regulatory processes in the cell are modulated by epigenetic mechanisms, and maintenance of or changes to the epigenome are recognised to have important roles in the regulation of gene expression during normal cell growth, fetal development and in the manifestation of diseases, including cancer (Bernal and Jirtle, 2010; Calvanese et al., 2009). Epigenetic changes may be inherited by daughter cells, but this not always the case.

2. There are three principal epigenetic mechanisms, namely changes to DNA methylation status, post-translational modifications to histones and RNA interference by non-coding RNAs, such as microRNAs (miRNAs) (Hamilton 2011) (see paragraph 11). Studies investigating the mechanisms that underpin the maintenance and modification of the epigenome indicate a substantial complexity in their regulation, which can be affected by nutritional, lifestyle and environmental factors. The fact that epigenetic processes are susceptible to perturbation by environmental and lifestyle factors is now well established and there are substantial efforts underway to evaluate the extent to which these changes contribute to public health effects and whether they should be routinely included in chemical risk assessment strategies (Marczylo et al 2016; EFSA 2016). Accordingly, it was considered appropriate for the Committees to hold a joint workshop to discuss the topic.

3. The heritability of some epigenetic changes gives rise to the possibility of transmission of effects to future generations. For the purpose of this report, ***multigenerational effects*** are those seen in exposed generations, including those that may have been exposed *in utero*, as offspring or gametes. ***Transgenerational effects*** are those seen in generations that have not been exposed, either directly to the substance under consideration or indirectly as offspring or gametes via parental exposure. For example, transgenerational effects can only be identified from the F3 generation when the parental generation (F0), in this case the mother, is exposed during pregnancy, as the mother (F0), the offspring *in utero* (F1) and gametes (F2) will all be exposed. However, if a non-pregnant animal (male or female) is exposed, transgenerational effects can be identified from the F2 generation as only the parent (F0) and gametes (F1) can be exposed (see Figure 1) (Skinner 2014). It is acknowledged that there is inconsistency in the terminology across the field, so they are defined here for clarity.

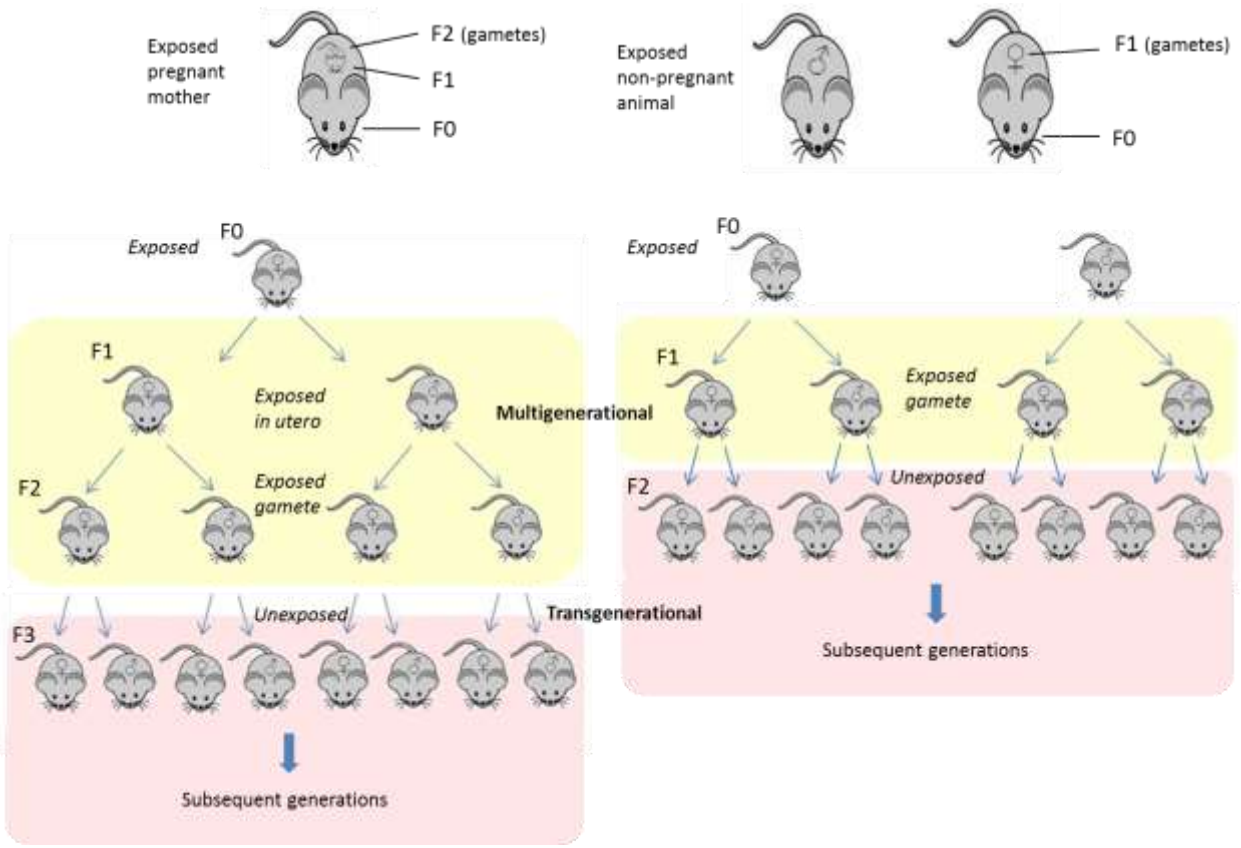

Figure 1. Schematic of multigenerational and transgenerational effects

Previous Committee considerations

4. The importance of epigenetic alterations has been considered by all three Committees previously. In October 2006 and again in June 2016, the COM examined the role of methylation status in transgenerational epigenetics. In particular, they looked at the potential for the fungicide vinclozolin to induce transgenerational effects in rats via the male line, following exposure of pregnant females (Anway et al 2005; Skinner et al 2013). The COM concluded that inconsistencies in the various studies (different animal strains, sampling points, routes of exposure) made interpretation difficult, as methylation patterns might be

expected to change 'naturally' over time in response to 'natural' changes in environmental exposure. It was noted by the COM that interactions between epigenetic changes and genotoxicity were possible, for example, epigenetic changes could exacerbate or antagonise a genotoxic effect (COM, 2006; COM, 2016a,b).

5. A preliminary evaluation of the role of epigenetics in carcinogenesis was undertaken by the COC in 2013. Arsenic and benzene were examined as examples of known human carcinogens that have epigenetic changes implicated in their mode of action (MOA) (Pilsner et al 2009; Bollati et al 2007). The overall conclusion was that it was possible that epigenetic changes contribute to carcinogenicity for arsenic and benzene, but that the role of epigenetic changes in their carcinogenic MOA needs further clarification. It was noted that '*epigenetic changes could be both causal for tumour development and the result of tumour development'* (COC, 2013).

6. In 2008, following the COM's discussion on the transgenerational effects of vinclozolin, the COT held a one-day workshop on the possible transgenerational effects of epigenetics more generally (COT, 2008). The overall conclusion was that there is reasonable evidence that epigenetic changes associated with environmental exposures during development can result in adverse effects in subsequent generations, although it is not clear whether transmission of acquired epigenetic changes occurs across generations in humans. It was also unclear how common transgenerational effects in animals are a result of epigenetic changes.

Joint Committee workshop

7. At the workshop, held in October 2017, delegates were asked to consider the overarching question '*Whether epigenetics should be used in chemical risk assessment'*, which framed the day's deliberations. Three presentations were given to provide background to the day and to stimulate thought and discussion.

8. Following the presentations, delegates were organised into breakout discussion groups, which focussed on the following questions:

- What is normal epigenetic variability and adaptation?
- How can epigenetic change be linked to adverse outcomes and adverse outcome pathways?
- What are the next steps to enable epigenetic change to be interpreted for incorporation in chemical risk assessment?

9. This statement summarises information from the speakers' presentations given at the workshop, the outcomes of the breakout group deliberations and the subsequent discussions and conclusions.

Overview and current awareness (Professor Tim Gant)

10. Epigenetic mechanisms can give rise to heritable change in phenotype without an associated change in genotype. This underpins many genetic processes that do not adhere

to normal gene expression patterns; for example, gene dosage effects are not seen in females, compared to males, despite the presence of two copies of the X chromosome.

11. Currently, three principal epigenetic mechanisms are known (Herceg 2007; Selbach et al 2008; Suganuma and Workman 2011):

- DNA methylation by modification of the cytosine base of DNA at the 5' position
- Histone modification by modifying the tails of the DNA histone proteins around which DNA is wound
- Perturbation of non-coding RNA species such as miRNAs that can affect the translation and degradation of messenger RNAs (mRNA)

12. Modification of cytosine by methylation is central to the regulation of gene expression, and changes in DNA methylation can form part of the cancer genome instability phenotype. Hypomethylation, often resulting in increased gene expression, is a common finding in neoplastic tissue whereas hypermethylation, for example of tumour suppressor genes, usually results in gene inactivation. Hydroxymethylation can also occur, which reverses the effects of methylation. During DNA replication, epigenetic marks (sites of cytosine modification), particularly methylation and hydroxymethylation of CpG sites that determine, in part, how the genome responds through regulation of transcription, are not copied onto the new strand (Herceg 2007).

13. Histone modifications can occur at the N-terminal 'tails' of histone proteins protruding from the nucleosomes. Such post-translational modifications include acetylation, methylation, ubiquitination and phosphorylation (Herceg 2007). Chromatin packaging can be influenced by these modifications of the histones, which results in a relaxation of chromatin and increased transcription (Suganuma and Workman 2011).

14. Most work on non-coding RNA species, as epigenetic modulators, has been on miRNAs. Their role is attributed to their inhibition of translation and/or degradation of target mRNAs, which in turn regulates gene expression. It has been demonstrated that a single miRNA species has the potential to repress protein synthesis from thousands of genes (Selbach et al 2008). Some miRNAs have considerable potential as biomarkers; for example to predict liver toxicity such as mir-122 and mir-192 (Wang et al 2009), although Rieswijk concluded that genotoxicity and non-genotoxicity probably cannot be accurately predicted based on miRNA profiles (Rieswijk et al 2016).

15. Methylation changes play an important role during gamete and zygote formation. A wave of demethylation in primordial germ cells removes sex specific marks during embryogenesis. In males, re-methylation within the primordial germ cells occurs before birth. In females, demethylation induces mitotic arrest during embryogenesis and re-methylation of the primordial germ cell occurs at puberty and triggers oocyte maturation. Epigenetic miRNA modifications, soon after fertilisation, are believed to stimulate gene transcription at the 8 cell stage, which is essential for fetal growth and differentiation (Tulay and Sengupta 2016).

16. It is known that epigenetic marks are not constant, and changes within the epigenome can fluctuate with age, lifestyle and exposure to environmental chemicals; this is known as

epigenetic drift. This drift may be a consequence of homeostatic, epigenetic adaptation and therefore may not necessarily imply cause and effect. Epigenetics may also explain Lamarckian inheritance theory, i.e. the idea that an organism can pass on phenotypic changes, acquired during its own lifetime, to its offspring (Skinner 2015).

17. There are several examples of chemically-induced epigenetic changes in human epidemiology studies (Marczylo et al 2016). For example, arsenic has been shown to alter global DNA methylation in human subjects (Pilsner et al 2007; 2009) and the drug valproate causes epigenetic reprogramming and histone deacetylation in human cells (Milutonovic et al 2007). However, the implication of these changes for adverse health effects is less clear.

18. One of the most examined aspects of epigenetic modifications is 'transgenerational' effects. Vinclozolin-induced transgenerational effects have been reported to occur via the male line, where epigenetic alterations are transmitted due to alterations in non-coding RNA extending to (at least) F3 generation rats (Schuster et al 2016). However, there are limitations in the studies investigating the effects of vinclozolin, such as the use of exceptionally high doses and the intraperitoneal route of exposure, which confound interpretation (COM, 2016b).

19. The difficulties in using the results from epigenetics studies in a regulatory context are numerous and complex. Unlike the genome of an individual that is the same throughout all somatic cell types, the epigenome of that individual differs between cell types, from cell to cell, and over time. So, whilst it is possible to study the genome in surrogate tissues (and species), studying the epigenome in **surrogate** tissues is unlikely to provide reliable data on the toxicity developing in a different target tissue or in the same tissue in a different animal species.

Evidence of human epigenetic responses to environmental exposures (Professor Jean Golding)

20. Trans- and multigenerational effects have been widely observed in human studies and are now being examined in terms of chemical-induced epigenetic alterations. The concept of multigenerational modulation of gene expression, for example via genomic imprinting, and the idea that a response of the parent to a physiological or social stress can modify offspring development, is now well established (Pembrey 1996; Jones et al 2005). It is believed that these modulations can explain the impact of nutritional status, stress or exposures to chemicals such as those resulting from smoking, on subsequent generations.

21. A number of large, longitudinal studies were presented in which various epigenetic parameters were assessed over time, and the impacts of lifestyle factors or chemical exposures in humans were examined. These included the Avon Longitudinal Study of Parents and Children (ALSPAC), the Överkalix study and the German 1916-18 famine study. It was noted that longitudinal studies of trans- and multigenerational effects are considered to be the 'gold standard'.

22. The Överkalix study examined the population of an isolated community in northern Sweden (164 men and 139 women, born in 1890-1920, and their 1818 children and

grandchildren) (Pembrey et al 2006). Historical records, including harvest outcomes and food prices, and smoking patterns, were used to investigate the impact of nutritional and smoking status on mortality and body mass index (BMI) of children and grandchildren. It was concluded that a grandson's health is influenced by pre-pubertal exposure of the paternal grandfather, and that a granddaughter's health is influenced by prenatal or infant exposure of the maternal grandmother.

23. The German 1916-18 famine study was also used to investigate the transmission of effects to subsequent generations. Famine during mid-childhood of the paternal grandfather and maternal grandmother was associated with higher mental health scores in grandsons and granddaughters, respectively (van den Berg and Pinger 2014). Kuzawa (2005) provides evidence that fetal nutrition triggers permanent adjustments in a wide range of systems and health outcomes, and speculates that these may be epigenetically modulated.

24. A series of studies examining smoking, DNA methylation, and potential effects in the offspring were presented (Cecil et al 2016; Küpers et al 2015; Miller et al 2014; Richmond et al 2015; Shorey-Kendrick et al 2017). In addition, epigenetic biomarkers of smoking-related effects have been investigated, including telomere length, 'epigenetic age' (an estimate of biological age based on changes in DNA methylation) and specific methylation sites (Horvath 2013; Simpkin et al 2016). Reese et al (2017) reported the DNA methylation score to be closely correlated with levels of cotinine in pregnant mothers; hence DNA methylation in newborn children was developed as a biomarker of sustained maternal smoking in pregnancy.

25. Attention was also drawn to several other factors that can alter methylation. For example, maternal obesity, maternal clinical depression and micronutrient supplementation may impact on future generations via altered histone methylation (Reynolds et al 2015). The importance of factors such as vulnerable ages, for instance, the periconception and *in utero* periods, and specific windows of susceptibility, were also highlighted (Silbergeld and Patrick, 2005).

Impact of xenobiotic-induced epigenome perturbations for safety assessment (Dr Jonathan Moggs)

26. The presentation focused on examining the key questions posed to workshop participants (see paragraph 8). It was suggested that there is a lack of knowledge on epigenomic 'normality' due to gene, cell, tissue, gender, strain and species specificity. Many different xenobiotics lead to dynamic epigenomic modifications, but most of these modifications are likely to be non-adverse as they accompany changes in gene expression underlying normal cellular responses and adaptation. Some induced perturbations of the epigenome may lead to adverse outcomes that may cause long lasting effects; and epigenetic responses to xenobiotics can precede overt toxicity phenotypes. Overall, the need to elucidate molecular mechanisms, to phenotypically anchor specific epigenomic perturbations, and to assess the potential for human translation of the effects was stressed.

27. The development of drugs that exert their therapeutic MOA via an epigenetic mechanism (e.g. anti-cancer drugs targeting chromatin and transcription factors) can provide

insight into the safety assessment of chemicals that induce epigenetic effects. Even in the pharmaceutical arena, there are currently no standards for addressing the safety of epigenetic targets. There will be a diversity of targets and mechanisms due to the complexity and intrinsic nature of epigenetic control of gene expression. A case-by-case approach to safety assessment, considering factors such as duration, schedule, reversibility, study endpoints, mechanism-based biomarkers and translatability, is required.

28. Some safety concerns for therapeutic epigenetic modifiers were identified, including short-term nuclear function effects (Olaharski et al 2006) and embryo-fetal toxicity, and multigenerational epigenomic changes via germline toxicities were highlighted (Erhardt et al 2003; Greenberg et al 2017). Additionally, molecular epigenomic reprogramming may result in delayed onset effects, long-lasting or permanent epigenomic changes in somatic cells, and/or lead to phenotypic effects such as morphological, functional or biochemical changes.

29. A number of case studies were presented that provided novel mechanistic insights such as:

- epigenetic changes being among the earliest events during non-genotoxic carcinogenesis, as misregulation in epigenetic regulatory proteins and aberrant expression of stem cell reprogramming genes may be associated with cancer aetiology and progression (Feinberg et al, 2006);

- activation of epigenetically imprinted non-coding RNAs (Lempiäinen et al 2013); and

- strain/species specificity and human relevance of epigenomic marks (Thomson et al 2016).

30. A second case study discussed integrating genetic and epigenetic data to support carcinogenicity risk assessment, using therapeutic fumarates as an example (Højfeldt and Helin 2016).

31. Evidence was also presented for multigenerational and transgenerational epigenetic perturbations by endocrine disrupting chemicals (Xin et al 2015); transgenerational actions of vinclozolin on sperm (Guerrero-Bosagna et al 2010); multigenerational epigenetic adaptation of the hepatic wound healing response (Zeybel et al 2012); and transgenerational environmental reprogramming of metabolic gene expression in mammals (Carone et al 2010).

32. Epigenomic atlases were presented, which give novel insights into cellular, tissue, gender, strain and species-specificity. These would enable critical assessment of human relevance for xenobiotic effector genes and pathways within *in vitro* and *in vivo* safety models.

33. The overall conclusions were that significant developments in methodologies for assessing epigenetic endpoints have been made, and that it is plausible to address this in safety assessment paradigms. The challenge is understanding the natural variability between strains, species, sex and age and what constitutes ‘healthy’ or ’diseased’. Epigenetic

inheritance may thus be a biological means for humans to adapt to changing environments and to transmit environmental information to offspring.

Committees' discussion questions:

What is normal epigenetic variability and adaptation?

34. There was a general consensus that, currently, not enough is known to be able to define 'normal epigenetic variability'. It was widely accepted that there are substantial differences between species, and significant variation between individuals within species, life/developmental stage and across organs/tissues. A large number of intrinsic factors, such as stress and nutrition/diet, are known to impact on 'normal' variability. Within the human population, other variables such as ethnicity and environmental factors (e.g. pollution) may also result in altered 'normal' patterns of epigenetic marks. It was recognised that a vast amount of information would have to be collated, from a range of species, ages, and from both sexes, to determine the extent of variability for all epigenetic marks. It was considered that the task of elucidating these nuances, and understanding their toxicological impact, will be too difficult an undertaking with the current level of understanding, and was not currently recommended.

35. It was considered important to understand what constitutes an adaptive change in epigenetics and what this represents in relation to what might be regarded as the 'normal' range. For example, it is known that there are age-dependent changes in epigenetics in humans that are considered to be adaptive, and there are also known multigenerational adaptations e.g. in famine situations when the body is programmed to famine status and the offspring are obese through an adaptive mechanism (Pembrey et al 2006); or resistance to chemically-induced liver damage in offspring of rats given hepatotoxic chemicals (Zeybel et al 2012). However, it was also recognised that there is a sizable gap in knowledge with respect to homeostatic adaptation and how to distinguish this from adverse effects. It was discussed as to whether specific classes of compounds known to induce epigenetic change could be used to examine the mechanisms which underpin the differences between homeostatic adaptation and adverse responses.

36. The epigenomic approaches outlined in the presentations are expected to be useful in establishing 'normal' ranges and the extent of normality of epigenetic marks. It was noted that specific microarrays are available that can be used to examine epigenetic changes in blood taken from human subjects during projects such as the ALSPAC. Similar microarrays could be developed for use in rodent studies.

37. With regards to investigating epigenetic changes within a risk assessment paradigm, it was noted that the variability between species is problematic and what constitutes an adverse response in one species may be an adaptive response in another. For example, gene imprinting, which has the potential to bring about significant effects across generations, varies between species. Furthermore, to understand epigenetic heterogeneity and what constitutes homeostatic adaptation or adverse responses, it would be important to examine the patterns of change as well as the extent and magnitude of change in different models.

How can epigenetic change be linked to adverse outcomes and adverse outcome pathways?

38. There are some known associations between epigenetic changes and the development of specific conditions (e.g. Beckwith-Wiedemann syndrome and Angelman syndrome), consequences of epigenetic errors during assisted reproductive technology (Niemitz and Feinberg 2004), and in some cancers (Herceg 2007). However, the mechanisms by which epigenetic changes result in adverse outcomes are not yet well understood. It is likely that different epigenetic changes, induced by different chemicals in different tissues (and/or species), will result in a wide variety of outcomes, only some of which will be adverse. Whilst it is assumed that a specific exposure may result in an epigenetic change, it is not yet possible to specify that a particular epigenetic change will lead to an adverse health outcome. However, it was generally agreed that the complexities of the various epigenetic processes, coupled with a lack of clarity as to what constitutes adverse changes, means that investigations using epigenetic endpoints may not always provide interpretable data.

39. Examining epigenetic change could be utilised to critique what is understood by a toxicological MOA to explore species differences and hence the relevance of findings to humans. Arsenic-induced tumours in rodents were considered to be an appropriate example of a carcinogenic MOA underpinned by epigenetic perturbation. Whilst this could readily be examined with regards to evaluating the relevance of arsenic induced effects in humans, the adverse outcome pathway (AOP) would only be applicable to other carcinogenic substances that share the same AOP.

40. Methods for examining epigenetics with a view to describing AOPs were discussed. It was suggested that integrated molecular and morphological testing could be used to assess the impact and reversibility of induced changes. For example, there are specific methylation inhibitors that could be used to investigate chemical-induced methylation changes.

41. Epigenetic methodologies are generally designed to be hypothesis generating. It was suggested that a framework could be established that could facilitate the interpretation and evaluation of these chemical-induced changes in a risk assessment scenario, e.g. whether a particular miRNA or histone modification is involved. Folate has a direct epigenetic target and could be considered as a model to understand the methylation AOP across species. However, it was considered that there is insufficient knowledge to enable such a framework to be constructed at present.

42. Issues surrounding species differences in epigenetic changes are known to be complex and, therefore, require careful consideration when designing and interpreting studies for generating information to derive AOPs. The use of *in vitro* test systems to investigate epigenetics was queried given the susceptibility of the epigenome of cultured cells to change, e.g. methylation changes are observed when cells are simply cultured or if cell culture conditions are altered. These factors all represent a challenge when attempting to tease out the differences between a toxicologically relevant epigenetic ‘signal’ and background ‘noise’ due to adaptations to environments that cells/animals find themselves in.

What are the next steps to enable epigenetic change to be interpreted for incorporation in chemical risk assessment?

43. The need to develop a better understanding of 'normality' was considered paramount. Whilst there is increasing knowledge of the mechanisms involved in epigenetic change, there is a need to elucidate specific mechanisms or pathways that are, or are not, relevant to humans. Investigative epigenetics research is generally carried out in mice. As studies conducted as part of chemical regulatory strategies predominantly use rats, it was suggested that rat models could be developed so that it would be possible to utilise regulatory studies when evaluating the impact of epigenetics in risk assessments. Selection of an appropriate rat strain should consider which is the most commonly used and whether the effects observed are relevant to humans. The development of genetically modified, knock-out rodent models, for example of specific histone acetylases, may facilitate the extrapolation of information from animals to humans.

44. It was suggested that a battery of techniques could be developed to provide a general screen, possibly *in vitro*, for epigenetic effects e.g. the use of marker genes associated with methylation. However, the difficulties of using cellular models are acknowledged and therefore, the design of a battery would be a challenging proposition.

45. With regard to studies in humans, there is a need for large, long-term prospective studies to establish and map what constitutes a background 'normal' epigenome and to investigate epigenetic-mediated phenotypic changes and their causes. A standardised protocol to examine human effects could be developed that could minimise variables or define specific circumstances, enabling investigators to pin down more precisely the nature and magnitude of induced epigenetic effects, and to predict outcomes of the changes. From this it may be possible to elucidate what constitutes an adverse from a non-adverse effect resulting from an epigenetic change. It was suggested that it may be possible to categorise substances in terms of the epigenetic changes they induce and from this a predictive framework could be devised.

Overarching discussions and overall conclusions

46. The overarching question 'Whether epigenetics should be included in chemical risk assessment?' provided the framework for the day's discussions. The following summarises the delegates consideration of this theme and the overall conclusions of the day:

- There is a need to increase our understanding of epigenetics, the potential for chemically-induced epigenetic changes and whether there is an impact on public health.

- Clearer definitions and characterisation of what represents 'normal' in the context of background variability of an epigenetic mark, and what constitutes an adverse or non-adverse epigenetic response are needed.

- Substances may cause effects in subsequent generations via an epigenetic mechanism. The COM will continue to review these potential effects on a regular basis.

- There is a need to understand how permanent epigenetic changes are and how these relate to subsequent changes, arising from mutations during reproduction, in later generations.

- A better understanding of how to extrapolate from *in vivo* data in animals to humans in subsequent generations is required.

- A better comprehension of species to species, and tissue to tissue, variability is required before attempts to interpret epigenetic changes in terms of human risk assessment can be undertaken with confidence.

- There is a particular need to understand dose-response relationships for epigenetic effects, which has to be addressed on a case-by-case basis. However, it is noted that at present we cannot make too many assumptions about what magnitude of a change in an epigenetic response is adverse.

- Whilst it is acknowledged that there are considerable uncertainties about the role of epigenetic changes and disease, it would be desirable to conduct careful and thorough prospective studies in humans, to look at associations between phenotype and epigenetic profiles, and the factors underlying these. There is also a role for studies that start by examining the adverse outcome and working backwards to the epigenetic changes.

- It was considered that the development of epigenetic biomarkers was of importance.

- It was agreed that much can be learned from epigenetic modifiers currently under development in the pharmaceutical field.

47. With regard to the inclusion of epigenetic evaluations within regulatory risk assessment frameworks, the following points are noteworthy:

- Given that epigenetic changes are basic biological responses and that there are high levels of uncertainty with regards to cause and effect of an epigenetic change, or what constitutes an adverse change, it is currently not clear how regulatory bodies could routinely use knowledge of epigenetics in risk assessments.

- It was established that a considerable amount of background information (on the epigenetic marks of concern) would be required before studies could be routinely incorporated into regulatory evaluations.

- It was generally agreed that epigenetic data could be submitted, e.g. in connection with regulatory submission, and this would facilitate the development of expertise in

exploring the impact of epigenetic changes. Accordingly, there is an appetite to generate a framework outlining experimental strategies and best practice.

48. Overall, it was concluded that:

- Evaluation of epigenetics presents a considerable challenge for risk assessment and there are currently insufficient data to identify epigenetic 'normality' and therefore to elucidate the potential impact of a chemical exposure. Despite this, there was a general opinion that current risk assessment practice is open minded and could expand to cover a range of epigenetic endpoints.

- Caution was advised with regards to classifying chemicals according to the way they regulate gene expression via epigenetic changes.

- Epigenetic data should be considered on a case-by-case basis, depending on what additional information is available. This may provide new/supporting evidence to confirm biological plausibility.

- To date no chemicals have been identified that exert their toxicity by a purely epigenetic mechanism. Public health protection is currently judged to be adequately provided by methods in which the ability of a substance to cause adverse outcomes, such as reproductive toxicity, cancer and genotoxicity, are assessed.

COC, COM and COT
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Professor Jean Golding: Centre for Child & Adolescent Health, Bristol Medical School, University of Bristol

Dr Jonathan Moggs: Novartis Institutes for BioMedical Research, Preclinical Safety, Translational Medicine

Glossary:

Adaptive response	The process whereby a cell or organism responds to a xenobiotic so that the cell or organism will survive in the new environment that contains the xenobiotic without impairment of function.
Adverse response	Change in morphology, physiology, biochemistry, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences.
DNA methylation	A reversible biochemical modification of DNA more or less universally present in organisms from bacteria to humans. Methyl groups can be enzymatically added to or removed from cytosine (C). It is associated with silencing of DNA sequences.
Epigenetic age	An estimate of biological age based on changes in DNA methylation at particular locations along the genome.
Epigenetic drift	Divergence of the epigenome as a function of age due to stochastic changes in methylation.
Epigenetic marks	Features not directly governed by the genetic code, which include methylation of DNA and covalent modification of histone proteins. The latter may also be tagged with methyl, acetyl, ubiquitin, phosphate, poly(ADP)ribose and other biochemical groups. These groups and their particular pattern of protein modification (e.g. mono-, bi-, tri-methylated at different amino acids and combinations of amino acids) modify the function of the tagged proteins and influence the way genes are expressed.
Epigenetics	The studies of heritable changes in gene function that occur without a change in the sequence of nuclear DNA and the processes involved in the unfolding development of an organism
Epigenome	The comprehensive collection of genome-wide epigenetic phenomena, DNA-methylation patterns, and chromatin modifications.
Epigenomic reprogramming	Resetting epigenetic marks so they resemble those of other cells from earlier developmental stages. This is of particular relevance for germline cells after the fusion of gametes when the genome is brought back into a kind of "zero-state" of gene expression.

Gene expression	The process by which the information in a gene is used to create proteins or polypeptides.
Genomic imprinting	The phenomenon whereby a small subset of all the genes in our genome are expressed according to their parent of origin.
Genotype	The particular genetic pattern seen in the DNA of an individual. It is usually used to refer to the particular pair of alleles that an individual possesses at a certain location in the genome.
Histone methylation	The modification of certain amino acids in a histone protein by the addition of methyl groups.
Histone modification	Covalent post-translational modifications to histone proteins including methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation, which regulate gene expression. The modifications made to histones can impact gene expression by altering chromatin structure.
Histone tails	A structural aspect of histones that are major targets for post-translational modifications of histones (see Histone modifications).
Hypermethylation	Increase in the methylation of cytosine-guanosine base pairs in regulatory regions of DNA.
Hypomethylation	The loss of the methyl group in 5-methylcytosine nucleotides in DNA. Hypomethylation can be used to describe the unmethylated state of specific nucleotides or as a general phenomenon affecting large parts of the genome.
Multigenerational effects	Effect seen in exposed generations, including those that may have been exposed in utero, as offspring or gametes.
Nucleosome	A repeating subunit of DNA packaging consisting of DNA wound in sequence around histone proteins.
Phenotype	The observable physical, biochemical and physiological characteristics of a cell, tissue, organ or individual, as determined by its genotype and the environment in which it develops.
Primordial germ cells	Highly specialised cells that are precursors of gametes, which, following meiosis, develop as haploid sperm and eggs that generate a new organism upon fertilization.
Somatic cells	Any biological cell that forms part of the body of an organism, excluding reproductive cells and undifferentiated stem cells.

Transgenerational effects	Effects seen in generations that have not been exposed, either directly to the substance under consideration or indirectly as offspring or gametes via parental exposure.

Abbreviations:

ALSPAC	Avon Longitudinal Study of Parents and Children
AOP	Adverse outcome pathway
BMI	Body mass index
COC	Committee on carcinogenicity of chemicals in food, consumer products and the environment
COM	Committee on mutagenicity of chemicals in food, consumer products and the environment
COT	Committee on toxicity of chemicals in food, consumer products and the environment
miRNA	MicroRNA
MOA	Mode of action
mRNA	Messenger RNA

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