This is a draft paper for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced

MUT/2018/04

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

Statement from a joint Committee workshop on the use of epigenetics in chemical risk assessment – updated first draft

1. In October 2017, the COC, COT and COM held a joint meeting. One of the topics discussed was "Whether epigenetics should be used in chemical risk assessment?"

2. This paper presents, in Annex A, a first draft statement on the outcomes of the discussion, updated following discussion at the COC meeting in November 2017 and with comments from the speakers and participants at the meeting. The document has been further amended with tracked changes from the COT meeting on 6th February 2018.

3. Annex B contains slides from all the discussion groups where the following questions were discussed:

- 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?

Questions for the Committee

4. Members are asked to consider:

   i. The structure and contents of the updated first draft statement

   ii. Are there any points from the discussion group slides that need to be further addressed within the draft statement?

Secretariat
February 2018

This is a draft paper for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced

MUT/2018/04 – Annex A

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

Statement from a joint Committee workshop on the use of epigenetics in chemical risk assessment – updated first draft

Updated first draft statement

NCET at WRc/IEH-C under contract supporting the PHE Secretariat
February 2018

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

MUT/2018/04

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 – updated first draft

Preamble:

The field of epigenetics research and the potential role of epigenetic changes in toxicology has 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 all 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 which provided a backdrop to the consideration of the question and delegates were later organised into breakout discussion groups. This statement summarises information from the presentations given at the workshop, the outcomes of the break-out group deliberations and the subsequent discussions and conclusions.

Introduction

1. Epigenetics is defined as modification in gene expression or cell phenotype caused by mechanisms other than a direct change in the DNA sequence. 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, foetal development and in the manifestation of diseases, including cancer (Bernal and Jirtle, 2010; Calvanese et al., 2009).

2. Three principle epigenetic mechanisms contribute to these alterations, namely changes to DNA methylation status, histone modifications and perturbation of microRNAs (miRNAs) (Hamilton 2011) (see paragraph 11). Studies investigating the mechanisms that underpin the maintenance and modification of epigenetics indicate a substantial complexity in their regulation, which can be affected by nutritional, lifestyle and environmental factors. Epigenetics is considered to be a critical factor in the regulation of gene expression and the onset and pathogenesis of diseases. The fact that epigenetic processes are susceptible to perturbation by environmental and lifestyle factors is now well established and there are substantive efforts to evaluate whether these changes represent a hazard to public health and need to be part of chemical risk assessment strategies (Marczylo et al 2016; EFSA 2016). Accordingly,

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

it was considered appropriate for the Committees to hold a joint workshop to discuss the topic.

3. For the purpose of this report, ***multigenerational effects*** are those seen in exposed generations, including those that may have been exposed *in utero*. ***Transgenerational effects*** are those seen in the first generation that are not exposed; if a mother is exposed during pregnancy, this will be the third subsequent generation (F3) but if the mother is exposed before pregnancy this will be the second subsequent generation (F2) (see Figure 1) (Skinner and Guerro-Bosagna 2011). It is acknowledged that other definitions for these exist.

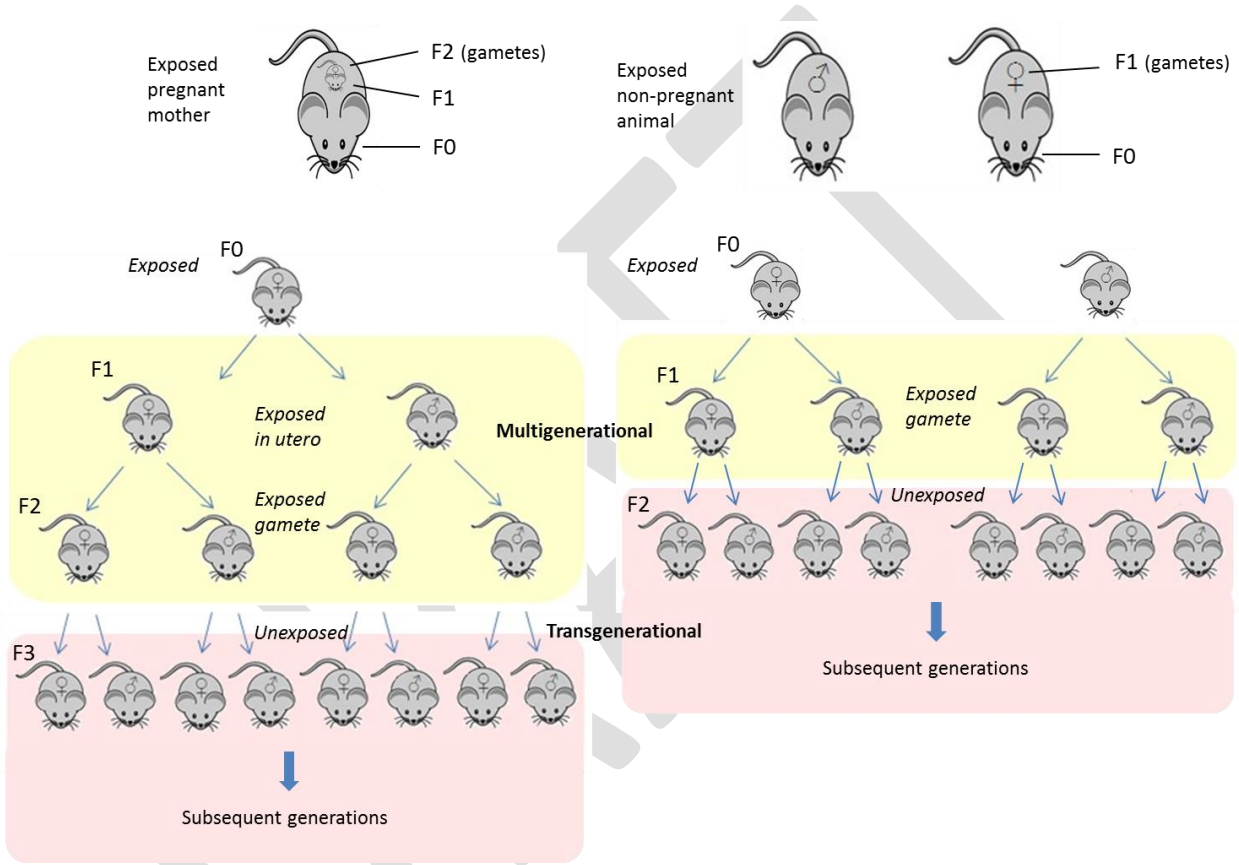

Figure 1. Schematic of multigenerational and transgenerational effects

Previous Committee considerations

4. The importance of epigenetic alterations has been considered by all three Committees previously. In 2008, the COT held a one-day workshop on the transgenerational effects of epigenetics (COT, 2008). The overall conclusion was that there is reasonable evidence that epigenetic changes that are 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.

5. A preliminary evaluation of the role of epigenetics in carcinogenesis was undertaken by the COC in 2013 (CC/2013/05 & CC/2013/06). 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 2001). 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.

6. In October 2006 (MUT/06/15) and again in June 2016 (MUT/16/05), the COM examined the role of methylation status in transgenerational epigenetics. In particular, they looked at the potential for the fungicide vinclozolin (VZ) to induce transgenerational effects in rats via the male line, following exposure of pregnant females (Anway et al 2005; Skinner et al 2013). It was concluded that inconsistencies in the various studies made interpretation difficult (different animal strains, sampling points, routes of exposure), as methylation patterns might be expected to change 'naturally' over time in response to 'natural' changes in environmental exposure. It was noted that interactions between epigenetic changes and genotoxicity were possible, for example, epigenetic changes could exacerbate or antagonise a genotoxic effect.

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 a backdrop to the day and to stimulate thought and discussion.

8. Following the presentations, delegates were organised into breakout discussion groups, and 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 break-out group deliberations and the subsequent discussions and conclusions.

Overview and current awareness (Professor Tim Gant)

10. Epigenetic mechanisms 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, why no gene dosage effect arising from a double copy of the X chromosome is seen in females.

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

11. There are three principle epigenetic mechanisms (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 messenger RNAs (mRNA) translation and degradation

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. Hypermethylation, for example of tumour suppressor proteins, usually results in gene inactivation. Hydroxymethylation can also occur, which reverses the effects of methylation and during DNA replication these marks are not copied onto the new strand (Herceg 2007).

13. Histone tail modifications can occur via acetylation, methylation, ubiquitination and phosphorylation. Chromatin packaging can be mediated by the modification of histones, which results in a relaxation of chromatin packaging and a more transcriptionally active state (Suganuma and Workman 2011) .

14. The role of miRNAs as an epigenetic modulator is attributed to inhibition of translation and/or degradation of target mRNAs, which in turn regulates gene expression. It has been demonstrated that a single miRNA can repress protein synthesis from thousands of genes (Selbach et al 2008). Some miRNA's are becoming established as biomarkers; for example to predict genotoxicity (Rieswijk et al 2016) or liver toxicity (Wang et al 2009).

15. Methylation changes have an important role during gamete and zygote formation. A wave of demethylation in primordial germ cells removes sex specific markers 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 start stimulation of gene transcription at the 8 cell stage, which is essential for foetal growth and differentiation (Tulay and Sengupta 2016). .

16. It is known that epigenetic markers are not constant and changes within the epigenome can fluctuate with age and exposures; this is known as epigenetic drift. This drift may be a consequence of hormetic, 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 characteristics that it has acquired during its 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).

18. One of the most examined aspect of epigenetic modifications are '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 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, 2016).

19. To conclude, a perspective on the challenges that epigenetics presents within a chemical regulatory environment was provided. The difficulties in using epigenetics in such 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. 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 target tissue.

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 induced epigenetic alterations. The concept of multigenerational modulation of gene expression, for example via genetic 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 these modulations can explain the impact of nutritional status, stress or other exposures such as smoking on subsequent generations.

21. A number of large, longitudinal studies were presented in which various epigenetic parameters were studied over time and the impacts of lifestyle factors or exposures in humans were studied. These included the Avon Longitudinal Study of Parents and Children (ALSPAC), the Överkalix study and the German 1916-18 famine. It was noted that the use of longitudinal studies to study trans- and multigenerational effects is 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, mortality and body mass index (BMI) of children and grandchildren. It was concluded that a grandson's health is influenced by pre-puberty 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 healthier mental health scores in grandsons and granddaughters, respectively (van den Berg and Pinger 2014). Kuzawa (2005) suggests there is evidence that foetal nutrition triggers permanent adjustments in a wide range of systems and health outcomes and it is speculated that these may be epigenetically modulated.

24. A series of studies examining smoking, DNA methylation and potential effects in offspring were presented (Cecil et al 2016; Kupers 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 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 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 depression and micronutrient supplementation may impact on future generations via methylation (Reynolds et al 2015). The importance of factors such as vulnerable ages 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 adaption. Some induced perturbations of the epigenome may lead to adverse outcomes that may cause long lasting effects; and epigenetic responses to xenobiotics can be prodromal to overt toxicity phenotypes. Overall, the need to elucidate molecular mechanisms, phenotypically-anchor specific epigenomic perturbations and assess the potential to human relevance 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 assessment 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.

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

28. Some safety concerns for therapeutic epigenetic modifiers were highlighted, including short-term nuclear function effects (Olaharski et al 2006) and embryo-foetal toxicity and multigenerational epigenomic changes via germline (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 or functional effects.

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

- epigenetic changes being the earliest events during non-genotoxic carcinogenesis, as mutations in epigenetic regulatory proteins and aberrant expression of stem cell reprogramming genes may be associated with cancer aetiology and progression (Feinberg 2006);
- activation of epigenetically imprinted non-coding RNAs (Lempiainien et al 2013); and
- strain/species specificity and human relevance of epigenomic biomarkers (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 (Guerreno-Bosagna et al 2010); multigenerational epigenetic adaption of 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.

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

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 inter-individuals within species 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 markers. 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 markers. It was considered that the task of elucidating these nuances and understanding their impact on evaluating epigenetic change will be too onerous difficult an undertaking 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 'normal' range. For example, it is known that there are age-dependent changes in epigenetics in humans that are considered to be adaptive and 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; or resistance to chemically-induced liver damage in offspring of treated rats. However, it was recognised that there is a sizable gap in knowledge with respect to 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 adaptive and adverse responses.

36. Epigenomic approaches are expected to be useful to establish 'normal' ranges and the extent of epigenetic markers. 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 an adaptive as opposed to an adverse response, it would be important to examine the patterns of change as well as the extent and magnitude of change in different models.

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

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), some cancers (Herceg 2007). However, the mechanisms which 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. It is noted that epigenetic changes are driven by genotype but that it is phenotypic change that results in an adverse effect. 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 a 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 or adaptive changes, means that investigations may not always provide meaningful answers.

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, it was not known whether the adverse outcome pathway (AOP) would be transferable to other carcinogenic substances.

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 not enough data areknowledge is available for 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

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

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 true epigenetic 'signal' and background 'noise'.

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 that generate 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 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 for epigenetic effects e.g. the use of marker genes associated with methylation. However, the difficulties 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 genotypic 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 adaptive or adverse effect of 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 days' 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.

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

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

- The potential for substances to cause effects in subsequent generations via an epigenetic mechanism was considered to be of concern.

- 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.

- There is a special particular need to understand dose-response relationships for epigenetic effects which has to be addressed on a case-by-case basis. However, it is advised that we cannot make too many assumptions about epigenetic factors, in particular what constitutes a 'normal' range or an induced change.

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

- It was considered that the development of methods to establish 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 adaptive or adverse change, it is currently not clear how regulatory bodies could use knowledge of epigenetics in risk assessments.

- It was established that a considerable amount of background information (on the epigenetic biomarker 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.

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

Accordingly, there is an appetite to generate a framework outlining experimental strategies and best practise.

48. Overall, it was concluded :

- Evaluation of epigenetics presents an enormous 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 practise 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.

- It was considered that current approaches to chemical risk assessment are effective at protecting human health. Knowledge tTo date indicates that there are no chemicals have been identified that exert their toxicity by a purely epigenetic mechanism and that other markers measures of toxicity provide enable appropriately protective risk assessmentshazard characterisation.

COC, COM and COT
[insert date]

Acknowledgements

The Committee would like to thank all the attendees of the workshop for their contributions, particularly the invited speakers:

Professor Tim Gant: PHE Centre for Radiation, Chemical and Environmental Hazards, Chilton

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

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

References:

Anway MD, Cupp AS, Uzumcu M, Skinner MK (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science, 3;308(5727),1466-9.

Bernal AJ and Jirtle RL (2010). Epigenomic disruption: the effects of early developmental exposures. Birth defects research. Part A, Clin mol teratol, 88, 938-944.

Bollati V, Baccarelli A, Hou L, Bonzini M, Fustinoni S, Cavallo D, Byun HM, Jiang J, Marinelli B, Pesatori AC (2007). Changes in DNA methylation patterns in subjects exposed to low-dose benzene. Cancer res, 67, 876-880.

Calvanese V, Lara E, Kahn A and Fraga MF (2009). The role of epigenetics in aging and age-related diseases. Ageing res rev, 8, 268-276.

Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, Meissner A, Weng Z, Hofmann HA, Friedman N and Rando OJ (2010). Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell, 143(7):1084-96.

Cecil CA, Walton E, Smith RG, Viding E, McCrory EJ, Relton CL, Suderman M, Pingault JB, McArdle W, Gaunt TR, Mill J, Barker ED (2016). DNA methylation and substance-use risk: a prospective, genome-wide study spanning gestation to adolescence. Transl Psychiatry, 6, 6-12.

COM (2016) Epigenetics: The transgenerational effects of vinclozolin. COT, COM and COC Annual Report 2016 pp 36-37. https://cot.food.gov.uk/sites/default/files/cotannualreport2016.pdf

COT (2008). Statement on the COT workshop on transgenerational epigenetics. COT statement 2008/03. http://cot.food.gov.uk/pdfs/cotstatementtransepi200803.pdf

EFSA (European Food Safety Authority) and Bahadori T, Bell D, Ceccatelli S, Corvi R, Hogstrand C, Munn S, Nilsson E, Spurgeon D, Vom Brocke J, Wray-Cahen D, Wright M, Binaglia M, Dorne JL, Georgiadis N, Germini A, Kass G, Robinson T, Rossi A, Schoonjans R, Terron A and Noteborn H (2016). EFSA Scientific Colloquium 22 - Epigenetics and Risk Assessment: Where do we stand? EFSA supporting publication, 13(12):EN-1129. 28 pp. doi: 10.2903/sp.efsa.2016.EN-1129

Erhardt S, Su IH, Schneider R, Barton S, Bannister AJ, Perez-Burgos L, Jenuwein T, Kouzarides T, Tarakhovsky A, Surani MA (2003). Consequences of the depletion of zygotic and embryonic enhancer of zeste 2 during preimplantation mouse development. Devel, 130 (8), 4235-48

Feinberg (2006). The epigenetic progenitor origin of human cancer. Nat Rev Genet, 7,21-33.

Greenberg MV, Glaser J, Borsos M, Marjou FE, Walter M, Teissandier A, Bourc'his D (2017). Transient transcription in the early embryo sets an epigenetic state that programs postnatal growth. Nat Genet, 49 (1), 110-118.

Guerrero-Bosagna C, Settles M, Lucker B, Skinner MK (2010). Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. PLoS One, 5(9), pii.

Hamilton JP (2011). Epigenetics: principles and practice. Digest dis, 29, 130-135.

Herceg Z (2007). Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. Mutagen, *22*, 91-103.

Højfeldt JW and Helin K (2016). Regional tumour glutamine supply affects chromatin and cell identity. Nat Cell Biol, 10(10), 1027-9.

Horvath, S (2013). DNA methylation age of human tissues and cell types. Genome Biol, 14, R115.

Jones R, Pembrey M, Golding J, Herrick D (2005). The search for genenotype/phenotype

associations and the phenome scan. Paediatr Perinat Epidemiol, 19(4), 264-75.

Küpers L, Xu X, Jankipersadsing SA (2015). DNA methylation mediates the effect of maternal smoking during pregnancy on birthweight of the offspring. Int J Epidemiol, 44(4),1224-37.

Kuzawa CW (2005). Integrated nutrition signal could lead to ‘‘intergenerational phenotypic inertia’’. Am J Hum Biol, 17, 5.

Lempiainen H, Couttet P, Bolognani F Müller A, Dubost V, Luisier R, Del Rio Espinola A, Vitry V, Unterberger EB, Thomson JP, Treindl F, Metzger U, Wrzodek C, Hahne F, Zollinger T, Brasa S, Kalteis M, Marcellin M, Giudicelli F, Braeuning A, Morawiec L, Zamurovic N, Längle U, Scheer N, Schübeler D, Goodman J, Chibout SD, Marlowe J, Theil D, Heard DJ, Grenet O, Zell A, Templin MF, Meehan RR, Wolf RC, Elcombe CR, Schwarz M, Moulin P, Terranova R, Moggs JG (2013). Identification of Dlk1-Dio3 imprinted gene cluster noncoding RNAs as novel candidate biomarkers for liver tumor promotion. Toxicol Sci, 131 (2), 376-86.

Marczylo EL, Jacobs MN, Gant TW (2016). Environmentally induced epigenetic toxicity: potential public health concerns. Crit Rev Toxicol, 46(8):676-700.

Miller LL, Pembrey M, Davey Smith G, Northstone K, Golding J (2014). Is the growth of the fetus of a non-smoking mother influenced by the smoking of either grandmother while pregnant? PLoS One, 9(2), e86781.

Milutinovic S, D'Alessio AC, Detich N, Szyf M (2007). Valproate induces widespread epigenetic reprogramming which involves demethylation of specific genes. Carcinogen, 28(3), 560-571.

Niemitz EL, Feinberg AP (2004). Epigenetics and assisted reproductive technology: a call for investigation. Am J Hum Genet. 74(4):599-609.

Olaharski AJ1, Ji Z, Woo JY, Lim S, Hubbard AE, Zhang L and Smith MT (2006). The histone deacetylase inhibitor trichostatin a has genotoxic effects in human lymphoblasts in vitro. Toxicol Sci, 93 (2), 341-347.

Pilsner JR, Liu X, Ahsan H, Ilievski V, Slavkovich V, Levy D, Factor-Litvak P, Graziano JH, Gamble MV (2007). Genomic methylation of peripheral blood leukocyte DNA: influences of arsenic and folate in Bangladeshi adults. Am J Clin Nutr, 86(4),1179-86.

Pembrey M (1996). Imprinting and transgenerational modulation of gene expression; human growth as a model. Acta Genet Med Gemellol (Roma), 45(1-2), 111-25.

Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjöström M, Golding J; ALSPAC Study Team (2006). Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet, 14(2):159-66.

Pilsner JR, Liu X, Ahsan H, Ilievski V, Slavkovich V, Levy D, Factor-Litvak P, Graziano JH, Gamble MV (2009). Folate deficiency, hyperhomocysteinemia, low urinary creatinine, and hypomethylation of leukocyte DNA are risk factors for arsenic-induced skin lesions. Environ Health Perspect, 117(2), 254-60.

Reese, SE, Zhao, S, Wu, MC, Joubert, BR, Parr, CL, Håberg, SE, London, SJ (2017). DNA methylation score as a biomarker in newborns for sustained maternal smoking during pregnancy. Environ Health Perspect, 125(4), 760-766]

Reynolds CM, Gray C, Li M, Segovia SA, Vickers MH (2015). Early Life Nutrition and Energy Balance Disorders in Offspring in Later Life. Nutrients, 7(9), 8090-111.

Richmond RC, Simpkin AJ, Woodward G, Gaunt TR, Lyttleton O, McArdle WL, Ring SM, Smith AD, Timpson NJ, Tilling K, Davey Smith G, Relton CL (2015). Prenatal exposure to maternal smoking and offspring DNA methylation across the lifecourse: findings from the Avon Longitudinal Study of Parents and Children (ALSPAC). Hum Mol Genet, 24(8):2201-17.

Rieswijk L, Brauers KJ, Coonen ML, Jennen DG, van Breda SG, Kleinjans JC (2016). Exploiting microRNA and mRNA profiles generated in vitro from carcinogen-exposed primary mouse hepatocytes for predicting in vivo genotoxicity and carcinogenicity. Mutagenesis. 31(5):603-15.

Schuster A, Skinner M, Yan W (2016). Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs. Environ Epigenet, 2(1), pii.

Selbach M, Schwanhäusser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N (2008). Widespread changes in protein synthesis induced by microRNAs. Nature, 455(7209), 58-63.

Shorey-Kendrick LE, McEvoy CT, Ferguson B, Burchard J, Park BS, Gao L, Vuylsteke BH, Milner KF, Morris CD, Spindel ER (2017). Vitamin C Prevents Offspring DNA Methylation Changes Associated with Maternal Smoking in Pregnancy. Am J Resp Crit Care Med, 196 (6), 745-755.

This is a draft statement for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced.

Silbergeld EK, Patrick TE (2005). Environmental exposures, toxicologic mechanisms, and adverse pregnancy outcomes. Am J Obstet Gynecol. 192(5 Suppl):S11-21.

Simpkin AJ, Hemani G, Suderman M (2016). Prenatal and early life influences on epigenetic age in children: a study of mother-offspring pairs from two cohort studies. Hum Mol Genet, 25(1),91-201.

Skinner MK, Guerrero-Bosagna C (2009). Environmental signals and transgenerational epigenetics. Epigenomics, 1(1),111-117.

Skinner MK, Guerrero-Bosagna C, Haque M, Nilsson E, Bhandari R, McCarrey JR (2013). Environmentally induced transgenerational epigenetic reprogramming of primordial germ cells and the subsequent germ line. PLoS One, 8(7), e66318.

Skinner MK (2015). Environmental epigenetics and a unified theory of the molecular aspects of evolution: a neo-lamarckian concept that facilitates neo-darwinian evolution. Genome Biol Evol. 27(5):1296-302.

Suganuma T, Workman JL. 2011. Signals and combinatorial functions of histone modifications. Annu Rev Biochem. 80:473–499.

Thomson JP, Ottaviano R, Unterberger EB (2016). Loss of Tet1-Associated 5-Hydroxymethylcytosine Is Concomitant with Aberrant Promoter Hypermethylation in Liver Cancer. Cancer Res, 15,76(10), 3097-108.

Tulay P, Sengupta SB (2016) MicroRNA expression and its association with DNA repair in preimplantation embryos. J Reprod Dev. 62(3):225-34.

Van den berg and Pinger (2014). A Validation Study of Transgenerational Effects of Childhood Conditions on the Third Generation Offspring's Economic and Health Outcomes Potentially Driven by Epigenetic Imprinting IZA 2014 Discussion paper 7999. Accessed in November 2017 http://ftp.iza.org/dp7999.pdf

Xin F, Susiarjo M, Bartolomei MS (2015). Multigenerational and transgenerational effects of endocrine disrupting chemicals: A role for altered epigenetic regulation? Semin Cell Dev Biol, 43, 66-75.

Zeybel M, Hardy T, Wong YK, Mathers JC, Fox CR, Gackowska A, Oakley F, Burt AD, Wilson CL, Anstee QM, Barter MJ, Masson S, Elsharkawy AM, Mann DA, Mann J (2012). Multigenerational epigenetic adaptation of the hepatic wound-healing response. Nat Med, 18(9), 1369-77.

This is a draft paper for discussion. It does not represent the views of the Committee and should not be quoted, cited or reproduced

MUT/2018/04 Annex B

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

Statement from a joint Committee workshop on the use of epigenetics in chemical risk assessment – updated first draft

Slides containing the output from the breakout discussion groups at the Joint COC, COM and COT meeting

Secretariat
February 2018

Group 1

COC, COM and COT Joint discussion of epigenetics

Discussion Group slides

Overarching question: Whether epigenetics should be used in chemical risk assessment

Please add a few bullet points to address each question for presentation by your Group Facilitator in the plenary discussion

Group 1

COC, COM and COT Joint discussion of epigenetics
Overarching question: Whether epigenetics should be used in chemical risk assessment

What is normal epigenetic variability and adaptation?

- We don't know
- Can use epic array (new) or 450 array to measure- but would also be required for rats and mice as reference species
- In vitro work is more challenging than in vivo.

- Adaptation – don't know enough
- Need to consider gene expression and stability
- Impact of ethnicity, sex, age- is adaptive capacity age dependent?
- Also how does it translate from one species to another?
- Also need to consider exposome

Group 1

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

- Use methylation inhibitors
- Crispr
- Humanised mice – e.g. PPARα study
- Make specific intervention
- Sensitivity of the timing of exposure- needs greater emphasis in regulatory assessments
- In many cases we don't need to know the epigenetic mechanism to regulate a chemical- it would not influence the margin of safety.

Group 1

COC, COM and COT Joint discussion of epigenetics
Overarching question: Whether epigenetics should be used in chemical risk assessment

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

- Generating a framework to establish when we need/want epigenetic information – joint committee effort

Group 1

Additional comments / notes

- Key Points:

Need more Knowledge.

Need to generate a framework to establish when we need/want epigenetic information – joint committee effort.

Group 2

COC, COM and COT Joint discussion of epigenetics

Discussion Group slides

Overarching question: Whether epigenetics should be used in chemical risk assessment

Please add a few bullet points to address each question for presentation by your Group Facilitator in the plenary discussion

Group 2

COC, COM and COT Joint discussion of epigenetics
Overarching question: Whether epigenetics should be used in chemical risk assessment

What is normal epigenetic variability and adaptation?

- Normal potentially changes over time
- Normal could differ between species and strains
- Difficult to extrapolate from animal to human
- Normal can depend on location and environment
- Difficult to define normal more work required

Group 2

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

- Arsenic possibly a model but may not be generalisable to other aspects normally considered by the Committees
- Epidemiological studies hypothesis generating but need to be tested
- Epigenetics as a tool for determining MOA
- Supporting data for MOA
- Could use to help evaluate human relevance
- microRNA to eliminate what wont happen

Group 2

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

- Identify if there is a problem
- Epigenetics can very specific e.g. insulin gene expression
- Supportive information on MOA (supports biological plausibility)
- Approach in the same way as systems biology
- Incorporate on a case by case basis

Group 2

Additional comments / notes

- Overarching answer:
- Not routine risks assessment
- Need to assess whether relevant before taking into account
- Need to ensure Agency/Department have adequate expertise to interpret

Group 3

COC, COM and COT Joint discussion of epigenetics

Discussion Group slides

Overarching question: Whether epigenetics should be used in chemical risk assessment

Please add a few bullet points to address each question for presentation by your Group Facilitator in the plenary discussion

Group 3

COC, COM and COT Joint discussion of epigenetics
Overarching question: Whether epigenetics should be used in chemical risk assessment

What is normal epigenetic variability and adaptation?

- There is currently not enough information to answer this question
- It will be dependent upon species, cell type, age, time point
- It is also likely to be environment specific increasingly complexity
- There should be individual questions for methylation, mRNA modification etc

Group 3

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

- Better linkage between epigenetic events and adverse outcomes
- Better understanding of adaptive effects
- Is there a role for starting with the adverse outcome and working backwards with well known compounds

Group 3

COC, COM and COT Joint discussion of epigenetics
Overarching question: Whether epigenetics should be used in chemical risk assessment

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

- Need to develop a better understanding of normal variation and how to measure it
- With 90 day studies potentially start dosing in utero and continue dosing after birth
- Battery of assays that will inform what compounds are epigenetic modulators for all epigenetic types ??

Group 3

Two key points

- Is there a role for starting with the adverse outcome and working backwards with well known compounds
- Battery of assays that will inform what compounds are epigenetic modulators for all epigenetic types ??

Group 4

COC, COM and COT Joint discussion of epigenetics

Discussion Group slides

Overarching question: Whether epigenetics should be used in chemical risk assessment

Please add a few bullet points to address each question for presentation by your Group Facilitator in the plenary discussion

Group 4

COC, COM and COT Joint discussion of epigenetics
Overarching question: Whether epigenetics should be used in chemical risk assessment

What is normal epigenetic variability and adaptation?

- Key issues:
- Variation across different populations, sexes, species, exposures – identification of susceptible populations
- Degree of variability (appears great)
- Permeability of changes? How do specific changes escape reprogramming?
- System capacity to adapt
- Level of sensitivity

Group 4

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

- With difficulty and expense!
- How do you know what to look for – which apical endpoints?
- A universal process that affects many systems
- Investigation of specific mechanisms/pathways under specific conditions would be useful (potential intervention/prevention)

Group 4

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

- Need to understanding specific mechanisms/pathways that are relevant to humans
- Level of sensitivity – use of carcinogenicity or other relevant models
- Stability? In vitro tests useful?

Group 4

Additional comments / notes

- What is happening already in our risk assessment? Already look at RA with an open mind
- Don't have enough information - yet
- Mixture effect/complications
- Would it actually change Public Health message – we "probably" are currently picking up adverse effects (creating more, unnecessary work)
- May have role to play in identifying potential trans/multigenerational toxicity

Group 5

COC, COM and COT Joint discussion of epigenetics

Discussion Group slides

Overarching question: Whether epigenetics should be used in chemical risk assessment

Please add a few bullet points to address each question for presentation by your Group Facilitator in the plenary discussion

Group 5

COC, COM and COT Joint discussion of epigenetics
Overarching question: Whether epigenetics should be used in chemical risk assessment

What is normal epigenetic variability and adaptation?

1. Perhaps we actually mean what is the baseline / control
   (The definitions are different.)
2. However far we map the epigenome – we still need to know how many changes and the magnitude that leads to adverse outcomes. Subsequently adaptation to these changes may mean that these changes do not lead to adversity.
3. The epigenome is different in different cell types, animal models, and differs with age, gender etc Do we need to know what is 'normal' in all of these tissues. This would be very demanding and there will be drift in control measurements (OS - with time I assume).
4. Which model should we relate to? Humans are **less or more** 'susceptible' and often unknown! There is no one animal model what we could use – same as with genotoxicity or other areas of toxicology.

CONCLUSION – DO WE REALLY NEED TO CARRYOUT SUCH AN EXTENSIVE EVALUATION OF ALL CELLS, TISSUES AND SPECIES? Perhaps not!

Group 5

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

1. Are all the outcomes adverse? Some changes are protective. But there are late onset adverse effects.
2. Epigenetic changes are driven by genotype. Therefore should we be looking at genotype and not complicate matters with epigenotype.
3. For exposures with known apical endpoints are sufficient why would you need to determine epigenetic changes. (e.g. a liver carcinogen)
4. Unable to answer this without some empirical data on past phenotype. How has the epigenetic change affected phenotype.
5. Current studies are retrospective, need some prospective studies.
6. Could use current cohorts as a source of tissue to study – human samples. This coupled with chemical evaluation would be powerful.

CONCLUSION WOULD NEED A VERY LARGE PROSPECTIVE STUDY TO FOLLOW ADVERSE EFFECTS, WHICH WOULD ALSO HAVE TO INCLUDE A RECOVERY PHASE. This is not possible or feasible

Group 5

COC, COM and COT Joint discussion of epigenetics
Overarching question: Whether epigenetics should be used in chemical risk assessment

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

1. More data through a well designed prospective study/studies
2. Is vinclosaline an exception??? Could we be chasing the exception rather than the rule?
3. We can learn from pharma examples
4. Need data from both rat and mouse. But which rat? The most commonly used strain.

Group 5

Additional comments / notes

- Is there a knock-out model?
- This was discussed by the group….
  - How can we use epigenetics as a screening tool. The model could be a cellular model.
-