Committee on MUTAGENICITY

MUT/MIN/2016/2

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

Minutes of the meeting held at 10.30 am on Thursday 16th June 2016 at the Department of Health in Room 137B Skipton House, Elephant and Castle, London, SE1 6LH.

Present:

Chairman:	Dr D Lovell
Members:	Dr C Beevers
	Dr G Clare
	Professor S Doak
	Dr S Dean
	Professor D Harrison
	Professor G Jenkins
	Professor D Kirkland
	Professor A Lynch
	Professor F Martin
Secretariat:	Dr O Sepai (PHE Secretary)
	Mr B Maycock (FSA Secretariat)
	Dr K Burnett (PHE Tox Unit)
	Mr S Robjohns (PHE Secretariat)
	Miss H Smith (PHE Secretariat)
Assessors:	Dr V Swaine (HSE)
	Mr S Fletcher (VMD)
In attendance:	Miss B Gadeberg (PHE COC Secretariat)
	For Item 4:
	Representatives from a company and consultancy for diflubenzuron

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ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed members, the secretariat and assessors. Mr B Maycock was substituting Dr D Benford as secretariat for the Food Standards Agency (FSA) and Miss B Gadeberg (PHE) was attending for the COC Secretariat. Professor D Harrison, the chair of the COC, was attending as an ex-officio member. The Chair also welcomed the assessors Dr V Swaine (HSE) and Dr S Fletcher (VMD).

2. Apologies for absence were received from Dr D Benford (Secretariat FSA), the Members Professor M O'Donovan and Ms P Hardwick, and from the assessors Dr H Stemplewski (MHRA) and Dr L Koshy (HSE).

3. Members were reminded of the need to declare any conflicts of interestand to ensure declarations of interests were kept up to date. No members declared a conflict of interest for the items on the meeting agenda.

ITEM 2: MINUTES OF MEETING ON 26 FEBRUARY 2016 (MUT/MIN/2016/1)

4. Members agreed the minutes subject to minor editorial changes.

ITEM 3: MATTERS ARISING

5. The committee was informed that two new vacancies (one for expert member and one for lay member) had been advertised and four reappointments were being processed. The members were asked to circulate the adverts to those who may be interested in the positions.

6. The COM also heard that a strategic review of the work undertaken at PHE is currently in progress and contributions had been requested from the chairs of the COC, COT and COM. The committee chairs would also no longer be attending the FSA General Advisory Committee on Science (GACS) as this committee had been disbanded and replaced by a Science Council.

7. FSA provided an update on cycloastragenol (a natural plant extract), which had been used in dietary supplements due to claims it has a beneficial effect on aging by stabilising the shortening of telomeres. The committee was reminded that cycloastragenol had been referred to the COC by the Advisory Committee on Novel Foods and Processes (ACNFP) as there were concerns over the claimed mechanism and carcinogenicity data. The COC supported those concerns and referred it to the COM for a view on the genotoxicity data. The COM agreed that the general package of data was negative, but recommended that an expert in telomere biology be consulted. The expert noted that teleomere shortening was a protective effect against DNA damage, so stabilising such teleomeres was potentially harmful. In addition, the expert suggested that mechanisms of telomere biology differ between shorter-lived rodent species and humans; therefore, the data provided may not be relevant to humans. Since those reviews, the applicant withdrew cycloastragenol from the approval process before the ACNFP could form an opinion. Consequently, cycloastragenol cannot be sold in the EU; however, it remains on sale

elsewhere, including the USA and China. The discussions on cycloastragenol by the ACFNP are recorded in the published minutes.

8. The Chair announced that this was the last meeting for Professor Anthony Lynch who had come to the end of his term. The Chair thanked Professor Lynch for all his excellent work over the years and the COM provided its best wishes for the future.

RESERVED BUSINESS

ITEM 4: GENOTOXICITY OF PARA-CHLOROANILINE – PRESENTATION

ITEM 5: DRAFT DISCUSSION PAPER: GENOTOXICITY OF PARA-CHLOROANILINE (MUT/2016/04)

OPEN SESSION

ITEM 6: ENVIRONMENTALLY INDUCED EPIGENETIC TOXICITY: POTENTIAL HEALTH CONCERNS- PRESENTATION

9. The role of methylation changes in mediating transgenerational effects was first examined by the COM in 2006 when the Advisory Committee on Pesticides (ACP) had requested an opinion on a paper investigating the pesticide vinclozolin. When the topic was presented at horizon scanning exercises (2013 and 2015), COM Members expressed an interest in examining the topic further, particularly with regards to the importance of examining these changes in risk assessment strategies. This topic was addressed by a presentation of work undertaken by the Toxicology Department at Public Health England (PHE) and by consideration of MUT/2016/05, which examined epigenetic experimental work on vinclozolin published since the 2006 COM review.

10. Dr Emma Marczylo provided a presentation to the COM of a recent literature review conducted by PHE and the associated substantial publication (Marczylo E et al., 2016. Critical Reviews in Toxicology. DOI 10.1080/10408444.2016.1175417), which evaluated environmentally induced epigenetic changes.

11. The review was split into 3 parts. The first part included an overview of the role of epigenetic mechanisms involved in the mammalian life cycle, particularly highlighting stages that might be vulnerable to epigenetic changes. The second and main section examined current evidence for environmentally-induced epigenetic toxicity from human cohort studies and animal (rodent) studies. This included adverse outcomes, such as reproductive toxicity, developmental toxicity, metabolic disorders and behavioural changes. The third

part of the review considered how potential epigenetic toxicity may affect public health. This included potential implications for regulatory toxicology.

12. Dr Marczylo noted there are various difficulties in interpreting these types of studies, such as the fact that epigenetic variation can be quite large in human and animal models. It is difficult to determine whether a change is normal and adaptive or adverse. It is also unclear whether a threshold needs to be exceeded for an adverse effect to occur. Eighteen human cohort and 20 rodent studies reported a lack of epigenetically-related toxicity. It was noted that these do not necessarily rule out an epigenetic mechanism of toxicity per se, but they do exclude a link between the specific epigenetic, exposure and/or adverse endpoints/markers investigated.

13. The PHE review suggested that there is a need to identify and investigate specific functional epigenetic mechanisms and biological pathways relevant to humans so that the risks of environmentally induced epigenetic toxicity to public health can be adequately assessed. The PHE review acknowledged that most studies involved a high, often single, dose in animals many orders of magnitude greater than human environmental levels of exposure. Also, a number of studies used routes of exposure that are not relevant to humans (e.g. intraperitoneal). Other factors to consider in evaluating potential relevance to humans, included differences in metabolism between humans and animal models; extrapolation from *in vitro* models to *in vivo*; timing of exposure in terms of susceptible life stages; and potential mixture effects (i.e. humans are exposed to an environmental mixture not just single chemicals). A robust, dose-dependent causal relationship between a specific environmental exposure, an epigenetic change and an adverse public health outcome would be required to classify a chemical as an epigenetic toxicant.

14. The COM was informed that consideration of epigenetic mechanisms was high on the agenda of the OECD and other expert groups. This indicated that there was a need to consider whether epigenetics should or could be included into regulatory chemical assessment. Also, epigenetics could potentially be used in exposure assessment i.e. where epigenetic changes act as indicators of exposure, but do not lead to adverse effects.

15. Regarding the future, Dr Marczylo suggested that more research was required. Improved human biomonitoring of chemical exposure may help determine the levels of chemicals that humans are exposed to environmentally before establishing whether relevant effects occur at these levels. There was also a need for improved molecular study designs to identify mechanisms for transgenerational effects using additional models (e.g. zebra fish), and to understand the normal variation of epigenetic change. Depending on such information, future test guidelines including epigenetic endpoints could be developed, which may be useful and could have benefits in terms of the 3Rs (reduction, replacement, refinement of animal use). For example, early epigenetic markers of adverse effect may result in a study being stopped early and no further testing being needed.

ITEM 7: EPIGENETICS: THE TRANSGENERATIONAL EFFECTS OF VINCLOZOLIN (MUT/2016/05)

16. Following the presentation, the COM was requested to provide its views or make suggestions that could help with future work in this topic. Also, to consider the potential implications for the COM from both the PHE review and from the data that had been provided relating specifically to vinclozolin (MUT/2016/05).

17. Members noted that a large amount of the chemical specific data on epigenetic changes related to vinclozolin. The COM considered that most of the studies on vinclozolin and other chemicals and epigenetic changes used very different methods (including different doses and different timing of doses) and different animal crosses. Some studies used very high doses and intraperitoneal administration that were not relevant to human environmental exposure. The inconsistency in the various studies made comparison difficult. Different time points of exposure could be important because methylation patterns might be expected to change 'naturally' over time in response to 'natural' changes in environmental exposure. Some of the results could be an artefact from the use of outbred animals and variation in the strains of animals used. However, it was acknowledged that much of the inconsistency could also be due to researchers investigating different or novel areas of research and not necessarily due to an underlying inconsistency in results or findings.

18. The COM considered that it would be important to identify and separate out the key epigenetic changes that could lead to adverse effects from the large 'natural' variation. It would be useful to identify what biomarkers or endpoints were important. There was a need for greater consistency in studies (e.g. timing of doses and routes of administration) and a standardisation of the protocols. There was also a need for reproducibility of studies (i.e. validation). Members suggested that there may be some currently available assays that could be used or adapted. It was also suggested that the input and involvement of wider scientific groups in addition to the COM could be beneficial.

19. Members agreed that it was not likely that existing test guidelines would be changed to include epigenetic endpoints in the foreseeable future. It was suggested that it would be useful to create a 'safe harbour' for epigenetic data that could receive data from industry, similar to that created for 'omics' data by the USA Food and Drug Administration. This would mean that data could be accumulated and made widely available for evaluation over time. This would allow regulatory bodies and industry to determine what endpoints and types of data may be useful and could be realistically obtained and added to existing toxicity studies. A repository would also be useful for existing data, which could include pre-clinical and clinical data.

20. The COM also noted that it was important to consider other groups that can be added to DNA, rather than just methyl groups (e.g. carboxyl, formyl etc.). Also, that there was a need to make further distinctions, such as between 5-methylation and 5- hydroxyl-methylation, because these changes may

represent different events (e.g. in terms of permanent effects). It was noted that methylation may up-regulate some genes; down-regulate others; and may have no effects on other genes.

21. Another important aspect to consider was whether there is a threshold for adverse epigenetic effects. Currently this was not known.

22. There was some discussion on what aspects of this area of research the COM could contribute to. Members considered that it would be important to identify any impacts that epigenetics could have on standard genotoxicity studies. Transgenerational heritable changes in the F3 generation were considered to have some overlap with the interests of the COM. It was considered that more subtle changes (e.g. seasonal, diurnal or effects more marked at particular life stages (i.e. non-permanent changes)) would be of less relevance to the COM. Any interaction between epigenetic change and genotoxicity would be more relevant to the COM. For example, if epigenetic changes could exacerbate or antagonise a genotoxic effect and whether this had any impacts on standard genotoxicity tests. It was also important to distinguish between epigenetic changes that can be re-programmed at a later time point and those that were resistant to re-programming.

23. Overall, it was considered that areas of epigenetics relevant to the remit of the COM would include any mechanisms for genetic damage and inheritance. Furthermore, there was a need for good reproducibility and validation of study findings before epigenetics could be considered in risk assessment and chemical regulation. It was suggested that a joint meeting of the three sister committees (i.e. the COM, COC and COT) on the topic of epigenetics would be useful.

ITEM 8: ANY OTHER BUSINESS

24. At the last OECD WNT meeting, the guidance document on revisions to OECD genetic toxicology Test Guidelines, previously seen by the COM, had been approved. The COM was also informed that there was a proposal for a test guideline on the microwell plate version of the Ames test.

25. One member expressed concern over four statements from the EFSA/ECHA regulatory reviews recently. There had been a statement that for *in vivo* genotoxicity, the intraperitoneal route of administration should be preferred to oral and inhalation because, firstly, it leads to by-pass of some first pass metabolism in the liver and therefore produces a more sensitive test. Secondly, that even if a test compound is detected in the plasma that it does not necessarily indicate that the target tissue in the bone marrow was also exposed to the test compound (i.e. in the micronucleus test). Thirdly, that even if it can be demonstrated that a test chemical has reached the bone marrow at a concentration that exceeds anticipated human exposure, it may not be considered adequate. This is because a higher exposure could be achieved in an *in vivo* comet or site of contact test. This would lead to the requirement for a further comet and site of contact tests to be conducted at a higher exposure. Fourthly, ECHA seemed to be insisting that in addition to the liver and the

duodenum that the glandular stomach should also be sampled as a second 'site of contact' tissue following oral administration. It was suggested that the COM should consider these recent regulatory genotoxicity testing requests at the next meeting.

26. The Health and Safety Executive (HSE) provided the COM with an update on glyphosate. The regulatory situation was ongoing. The EU was considering an application for renewal of the regulatory approval of the herbicide glyphosate. There was a proposal to extend the approval of glyphosate for 6 months while ECHA considered its final decision on its classification. Consideration on the extension of approval would take place on the 24th June. A proposal to amend the harmonised classification and labelling of glyphosate by the German Competent Authority was published on the ECHA's website for a 45 day public consultation period. Germany was not proposing to classify glyphosate as a germ cell mutagen or carcinogen, but the ECHA welcomed comments on thesehazard classes.

ITEM 9: DATE OF NEXT MEETING

27. 20th October 2016.