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

Preface

Forward by David Lovell - Chair

I am pleased to present this report, which provides a summary of the work of the Committee on Mutagenicity (COM) during 2016. The COM would be happy to receive any feedback from readers of this report.

The Committee on Mutagenicity (COM) provides advice on potential mutagenic activity of specific chemicals at the request of UK Government Departments and Agencies. Such requests generally relate to chemicals for which there are incomplete, non-standard or controversial data sets for which independent authoritative advice on potential mutagenic hazards and risks is required. Recommendations for further studies are, on occasions, made.

The Committee also advises on important general principles and on new scientific work related to the assessment of mutagenic risk and makes recommendations on mutagenicity testing. The membership of the Committee, declarations of their interests, agendas and minutes of meetings, and statements are all published on the internet. https://www.gov.uk/government/organisations/committee-on-mutagenicity-of-chemicals-in-food-consumer-products-and-the-environment

During 2016, the Committee reviewed a number of topics: the genotoxicity of parachloroaniline, assays used to evaluate germ cell DNA integrity, germ cell Adverse Outcome Pathways (AOPs) and a scoping paper on human germ cell mutagens. It discussed recent work on epigenetics and the potential transgenerational effects of Vinclozolin. It commented upon a systematic review on the health effects of emissions to air from municipal waste incinerators. It began a consideration of new quantitative approaches being proposed for the assessment of genotoxicity data. The Committee also carried out its annual Horizon scanning exercise, identifying a number of potential topics for future work. The COM is interested in obtaining information from Government Departments on how its advice is acted upon.

Throughout 2016 the COM continued to take an active interest in the work of the OECD (Organisation for Economic Cooperation and Development) on test guidelines. It commented on the OECD's review of old test guidelines (TGs) and the development of new TG's. It also commented on the OECD's Guidance Document on Revisions to OECD Genetic Toxicology Test Guidelines. The COM also discussed the possible implications of Brexit on its work and noted that there was uncertainty in how this may affect the regulatory environment and the UK's relationship with international organisations.

I am again grateful for the support of the secretariat and the Department of Health Toxicology Unit, who maintained their usual high standard of work despite the difficulties and uncertainties throughout the year and to the members of the committee for their expert advice and support throughout the year.

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COM Evaluations

MUT/2016/01 Assays used to evaluate germ cell DNA integrity in human fertility investigations

COM had previously considered germ cell mutation assays, the paternal age effect (an increase in mutations in aging human sperm) and a paper on radiation induced transgenerational effects. As part of the review the suggestion that air pollution should be classified as a human germ cell mutagen was noted and it was decided to perform a review of the literature to examine this claim. During the literature review it was noted that many studies of the effects of air pollution utilised assays for DNA integrity developed for use in assisted reproductive technologies (ART). Their use as markers of DNA damage in human sperm had not been validated and therefore it was considered appropriate for the COM to assess these assays before addressing the claim that air pollution is a germ cell mutagen.

The paper provided an overview of the sperm chromatin structure assay (SCSA) and the TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling) assays and their potential for investigating germ cell mutagenesis in humans. It was noted that both the SCCA and the TUNEL detect DNA strand breaks and therefore should be considered only as indicator assays. It was considered that they did not inform on the consequences of the DNA damage; whether they lead to a mutation or apoptosis; or whether damage would be repaired. It was not clear whether the reported reduced fertility was due to a genotoxic or toxic effect. It was noted that the two assays measured different types of DNA stand breaks and may not be directly comparable. It was possible that the observed DNA fragmentation could have arisen as a result of chemical induced oxidative stress, apoptosis, or another process not involving genotoxicity. There also appeared to be a relatively high background level which would make it difficult to detect a chemically induced increase in DNA fragmentation. Furthermore, it was not clear at what point in spermatogenesis the DNA damage occurs.

The COM considered that these assays may provide some evidence of chemically induced DNA damage, but there were a number of uncertainties which made both the SCSA and the TUNEL assays difficult to interpret in terms of germ cell mutagenicity. For example, there was a lack of consistency between some of the data and the test methods used; uncertainty over the underlying biology leading to the formation of DNA strand breaks and resultant downstream effects; a large variation in background levels and a lack of validation of the test methods. It would be useful to harmonise these methodologies and for the validation of these assays to be undertaken prior to their use in evaluating human germ cell mutagenicity. Accordingly, COM were not able to corroborate the conclusion made by DeMarini (2012 - Environ. Mol. Mutagen 53: 166-172), that air pollution is a human germ cell mutagen.

MUT/2016/02 Germ cell adverse outcome pathways

COM had been made aware of recent papers by a group from Health Canada regarding adverse outcome pathways (AOP) for germ cell endpoints (Yauk et al. 2015 Environ.Mol.Mut 56(9) 724-50 and Marchetti et al. 2015 Environ.Mol.Mut 57(2) 87-113) and these were evaluated as part of the ongoing review of germ cell mutagenesis. An individual AOP is developed for a specific molecular initiating event, is not chemical specific and has key toxicological effects, which should be measurable.

The DNA alkylation AOP (Yauk et al., 2015) focused on premeiotic germ cell DNA alkylation using ethylnitrosourea as a model alkylating agent. Unique features of germ cells suggest that they should be considered separately from somatic cells. The AOP makes the assumption that the processes of DNA repair and damage are conserved across eukaryotic cells. The tubulin binding AOP (Marchetti et al. 2015) used colchicine as a model example, the majority of evidence is generated from rodents. It was noted that benzimidazoles induce this AOP.

COM agreed that the two AOPs provided were very specific and more qualitative than quantitative but provide a useful framework for capturing and clarifying information obtained from systems biology approaches. They also provide frameworks to aid in the communication of mode of actions, but further development was required before they could be used in chemical safety evaluation. It was noted that one of the main difficulties was that there was no consensus on terminology across toxicology disciplines which would need to be addressed; it was noted that systems biology may facilitate this, as it already had a number of agreed terms.

COM agreed that currently, AOPs could not be used to evaluate mixtures of chemicals or in risk assessment. It was noted that COM 2007 statement on benzimidazoles, where a 'common mechanism' of toxicity had been identified used terminology a little different to that used in the AOP but that there were sufficient similarities and that the statement remained valid and did not need updating. It was agreed that COM would keep a watching brief on the development of AOPs for mutagenicity.

MUT/2016/04 Draft discussion paper: genotoxicity of parachloroaniline

RESERVED BUSINESS

MUT/2016/05 Epigenetics:

Transgenerational epigenetics 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. The topic was raised again during a Horizon scanning exercises and the COM expressed an interest in examining the topic further, particularly with regards to the impact on risk assessment strategies.

Dr Emma Marczylo (PHE), presented details and discussion of a PHE a recent literature review and associated publication (Marczylo E et al., 2016. Critical Reviews in

Toxicology XXZXX) which evaluated environmentally induced epigenetic changes. Firstly she addressed the role of epigenetic mechanisms involved in the mammalian life cycle, particularly highlighting stages that might be vulnerable to epigenetic changes; Dr Marczylo also 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.

Regarding the future, Dr Marczylo suggested that more research was required. Improved human bio-monitoring 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.

Epigenetics: The Transgenerational Effects Of Vinclozolin (MUT/2016/05)

The COM also considered a paper which provided an overview of epigenetics and studies specifically investigating the transgenerational effects of vinclozolin, which had been published since the last review (MUT/2016/05). A number of studies which had demonstrated a variety of vinclozalin induced effects using a dosing protocol of high intraperitoneal doses (100 mg/kg/day) to pregnant rats on days 8-14 of gestation were evaluated together with others using which had aimed to examine these findings. The COM noted that the epigenetic changes observed following the use of inconsistent methods (including different doses and different timing of doses) and different animal crosses made comparison difficult. The studies using very high doses and intraperitoneal administration were not considered to be relevant to human environmental exposure. Different time points of exposure could be important because methylation patterns change 'naturally' over time in response to environmental pressures. It was noted that some of the results could be an artefact from the use of outbred animals and variation in the strains of animals use. Furthermore, inconsistencies may be the consequence of researchers investigating specific or novel aspects of the research and not necessarily due to an underlying inconsistency in results or findings.

COM noted that it will be important to identify and separate out the key epigenetic changes that could lead to adverse effects from the large 'natural' variation. The identification of epigenetic biomarkers and endpoints was also considered important. There is a need for greater reproducibility and consistency within studies (i.e. validation) and COM suggested that some currently available assays could be used or adapted, although it was 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 could be made available, facilitating a broad evaluation which could allow regulatory bodies and industry to determine what endpoints and types data may be useful and which could be realistically obtained and added to existing toxicity studies.

The COM also noted that it was important to consider other chemical groups that can be added to DNA, rather than just methyl groups (e.g. carboxyl, formyl etc.) and that further distinctions, such as between 5-methylation and 5- hydroxyl-methylation, should be made. It was noted that epigenetic changes may up-regulate some genes; down-regulate others; and have no effects on other genes. It was noted that it was currently unknown whether there is a threshold for adverse epigenetic effects. COM considered that it would be important to identify any impacts that epigenetics could have on standard genotoxicity studies.

Overall, it was considered that areas of epigenetics relevant to the remit of the COM include potential mechanisms for genetic damage and inheritance. There was a need for validation of studies before epigenetics could be considered in risk assessment and chemical regulation.

MUT/2016/06 Systematic review on the health effects of emissions to air from municipal waste incinerators

RESERVED

MUT/2016/07 Quantitative approaches to the assessment of genotoxicity data.

COM were aware of work being conducted by a number of groups developing quantitative approaches to assessing genotoxic dose responses. The topic was addressed in a special issue of Mutagenesis published in June 2016 following an ILSI/HESI Genetic Toxicology Technical committee (GTTC) and European Environmental Mutagen Society /UKEMS workshop held in Lancaster in July 2014. The International Workshop on Genotoxicity Testing (IWGT) working group on Quantitative Genetic Toxicology Risk Assessment (the QWG) had also published the outcome of its discussions and consensus views.

The COM considered a scoping paper outlining these current approaches and evaluated the potential for data, from *in vivo* genotoxicity studies, to be used in a margin of exposure (MoE) approach to risk assessment, similar to that utilised in the interpretation of carcinogenicity data. A presentation was given by Dr George Johnson from Swansea University who presented some of the work that had been undertaken by ILSI/HESI GTTC and IWGT groups on these quantitative approaches. The presentation covered the derivation of Points of departure (POD) using a variety of metrics; the No Observed Genotoxic Effect Level (NOGEL), the Breakpoint dose (BPD), the Slope transition Dose (STD) and Benchmark Dose (BMD); and how PODs could be used to determine human exposure levels expected to present a low or negligible risk to health. A number of case studies were considered, including *in vivo* genotoxicity data sets for alkylating agents and benzo(a)pyrene. Consensus was reached by the study group that use of the BMD was the preferred option. It was noted that there are currently two approaches software. The US Environmental Protection Agency (EPA) BMD uses the best transformation of the response data for analyses, whereas the Netherlands National Institute for Public

Health and the Environment (RIVM) PROAST model uses the default assumption of a log-normal distribution. Furthermore, the Benchmark Dose Response (BMR) uses an increase relative to a negative control either by one standard deviation (US EPA) or a percentage (e.g. 5 or 10%) increased response (RIVM PROAST).

The COM agreed that there had been a change in the quality of available *in vivo* genotoxicity data (e.g. more endpoints, tissues and dose groups) and significant developments in dose response modelling that allow *in vivo* genotoxicity data to be analysed quantitatively rather than only qualitatively, but that the analysis needed be conducted on good quality and consistent data to be informative. Aspects that needed to be considered in terms of risk assessment included what test systems and endpoints were the most suitable (e.g. gene mutations or micronuclei), what tissues should be analysed, what critical effect size should be used (e.g. $BMDL_{05}$ or $BMDL_{10}$), and what BMR values were needed for each genotoxicity endpoint. It was also agreed that if quantitative dose-response analysis of *in vivo* genotoxicity is developed and becomes accepted as an approach to estimate human cancer health risks, then there must be confidence that the approach is sufficiently precautionary and protective of health. It was anticipated that quantitative approaches to genotoxicity data should be considered further by the COM at future meetings.

Horizon Scanning

Informal discussion (Oct 16) Reviewing ecological screening methods for the conduct of genotoxicity test on environmental pollutants.

HORIZON SCANNING

The COM undertakes an annual 'Horizon Scanning' exercise, which provides an opportunity for Members and assessors from Government Departments/Agencies to discuss and suggest topics for further work.

OECD GENOTOXICTY TEST GUIDELINES UPDATE.

The Committee continue to be updated and comment on, the review of old test guidelines (TGs) and the development of new TG's.
The Committee also commented on the Guidance Document on Revisions to OECD Genetic Toxicology Test Guidelines.

Guidance statements
None