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

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Preface

I am pleased to present this report, which summarises the work of the Committee on Mutagenicity (COM) during 2015.

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. Frequently recommendations for further studies are made.

The Committee also advises on important general principles and new scientific discoveries associated with the assessment of mutagenic risk and makes recommendations on mutagenicity testing. The membership of the Committee, 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 2015, the Committee published a statement on the mutagenicity of alcohol (ethanol) and its metabolite acetaldehyde which provided an update on information published on the topic between 2000- 2014 together with a paper on the potential role of oxidative damage as a mechanism of the genotoxicity of alcohol. This work was carried out to support the Committee on Carcinogenicity (COC)'s review of alcohol-induced carcinogenicity.

The COM considered whether there was evidence for a threshold for mutagenicity for Chromium VI and commented, at the request of the COC, on potential genotoxicity issues associated with the novel food ingredient Cycloastragenol.

The Committee carried out its annual Horizon scanning exercise, identifying a number of potential topics for future work, and as a part of this, had a presentation and discussion of the present status of 3D tissue models. The Committee also considered scoping papers on the potential effect of age on mutation rate and the evidence for transgenerational effects.

Throughout 2015 the COM continued to take an active interest by commenting on the reviews of the OECD (Organisation for Economic Cooperation and Development) Guidelines for Genotoxicity Testing. The COM also underwent a triennial review of its role and activities undertaken for the Department of Health and provided input into the PHE review.

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 they experienced through staff shortages and reorganizations and to the members of the committee for their expert advice and support throughout the year.

Dr D Lovell Chair
PhD BSc (Hons) FBS CStat CBiol CSci

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COM Evaluations

STATEMENT ON THE USE OF MUTATION SPECTRA IN GENETIC TOXICOLOGY: MUT/2015/S1

The term 'mutation spectra' (MS) refers to the composite of the number, types and sites of all mutations observed in a given sequence. It is also more loosely used in referring to the number and types of mutation found or even the main type of mutation observed (e.g. GC to AT transversions).

The COM reviewed a selection of papers considered to be a representative cross-section of studies examining mutational fingerprints and hotspots for mutation following carcinogen exposure in 2014. From these evaluations and discussions, a statement was generated and is available:

REVIEW OF THE MUTAGENICITY OF ALCOHOL

The potential role of oxidative damage in alcohol's mutagenic and carcinogenic mode of action

As part of the review of the alcohol and mutagenicity review, the potential role of oxidative damage in alcohol's mutagenic and carcinogenic mode of action was considered. The Committee agreed that the hypothesis that alcohol-induced oxidative stress could be important in alcohol induced liver disease and carcinogenesis was plausible. Reactive oxygen species generated from oxidative metabolism or inflammatory processes could give rise to lipid peroxidation products, which may lead to subsequent mutagenic adducts. Alcohol-induced mechanisms that could lead to the oxidative damage to DNA, include the generation of reactive oxygen species and induction of CYP2E1.

The COM noted that ethanol consumption can result in the induction of CYP2E1, primarily in the liver, but also in other tissues, such as the oesophagus and intestine. It was agreed that it was plausible that the induction of CYP2E1 enhanced the metabolism of alcohol to acetaldehyde; the generation of reactive oxygen species; and adduct formation. A correlation between CYP2E1 levels and DNA etheno adducts had been demonstrated in animal models and humans. It was noted that an association between CYP2E1 polymorphisms and alcoholic liver disease/alcohol induced carcinogenesis was not well defined and appeared to be weak.

Overall, Members agreed that oxidative damage to DNA was a plausible hypothesis and mode of action for genotoxicity and carcinogenicity for alcohol and its metabolite acetaldehyde, but that further evidence was required.

STATEMENT ON THE MUTAGENICITY OF ALCOHOL (ETHANOL) AND ITS METABOLITE ACETALDEHYDE: UPDATE ON INFORMATION PUBLISHED BETWEEN 2000-2014 (MUT/2015/S2)

The COM considered an updated review of the mutagenicity of alcohol and its primary metabolite acetaldehyde following a request from COC to support its on-going review of alcohol induced carcinogenicity in 2014. This review provided insight to COC regarding possible mechanisms of cancer causally associated with the consumption of alcoholic drinks.

A statement summarising these papers and the discussions, including those pertaining to the potential oxidative mechanisms, was published and is available MUT/2015/S2

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https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/490582/COM_2015_S2_Alcohol_and_Mutagenicity_Statement.pdf

CHROMIUM VI –

The Environment Agency asked Public Health England whether a paper published by Thompson et al., 2013a (A chronic oral reference dose for hexavalent chromium-induced intestinal cancer. Journal of Applied Toxicology. 34(5): 525-36) and associated work by that group had demonstrated a threshold for the genotoxicity of Chromium (VI) following oral exposure. This paper contends that the mode of action (MOA) for intestinal neoplasms following oral exposure to Cr(VI) involves cytotoxicity and not mutagenicity. Therefore a threshold for carcinogenicity is claimed, and that it is appropriate to derive an oral reference dose (RfD/TDI) from intestinal tumour data. This is contrary to the current UK position that affirms the mutagenic potential of Cr(VI) via oral exposure and therefore, that there is no threshold its carcinogenicity. Members were asked for their opinion on the evidence for a threshold for the mutagenicity of Cr (VI) following oral exposure.

Members considered a number of studies that contributed to the evaluation of the MOA of Cr(VI) induced intestinal neoplasms and overviews of available data relating to the genotoxicity of Cr (VI), including summaries from the European Food Safety Authority (EFSA 2014) and the US Agency for Toxic Substances and Disease Registry (2012). Members noted that there were two separate questions that could be considered. One was whether data provided by Thompson et al., and O'Brien et al., demonstrated a threshold for the mutagenicity of Cr(VI) via oral exposure and the other was whether there was a potential threshold due to the conversion of Cr (VI) to the non-mutagenic Cr (III) in the gastrointestinal tract. It was decided that the discussion would focus on the former.

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Members commented on a number of practical or methodological aspects of the studies used to substantiate the claim for a cytotoxic MOA. The selection of K-*Ras* as the most sensitive marker for mutagenicity was queried, and it was suggested that other key mutated genes and K-Ras in other tissues (i.e. the small intestine) could be investigated. It was agreed that a key weakness of the investigation was the lack of a suitable positive control, one that could be anticipated to act via the same mechanism (e.g. a direct acting alkylating agent). Furthermore, it was noted that it would be difficult to detect an induced mutagenic effect as the reported background frequency of K-*Ras* mutation was very high. Members were not convinced that the dose related increase in micronuclei (MN) in the duodenal villi was solely due to cytotoxicity. It was felt that the use of the paraffin sections as described had insufficient sensitivity for identifying MN.

Members were not convinced by the arguments presented in the paper which claimed there were no correlations between K-*Ras* mutations and Cr-DNA binding and that this did not represent pre-mutagenic DNA damage. It was agreed that the authors had not sufficiently negated concern for potential mutation following Cr-DNA binding.

Overall the committee agreed that whilst the hypothesis was plausible, there were limited data to demonstrate a threshold for genotoxicity for Cr (VI) and it was not convinced that there was a clear negative result for genotoxicity at low doses.

GERM CELL MUTAGENESIS

A paper on germ cell mutagenicity testing was considered by the Committee in 2013 and it had concluded that further validation work was needed before newly developed germ cell assays could be incorporated into general genotoxicity testing. During the horizon scanning exercise in June 2015, Members considered a recent suggestion that there is a need to investigate and

advise on the existence and implications of human germ cell mutagens, in a manner similar to that undertaken by IARC for human carcinogens. In addition, it was noted that the advent of high throughput sequencing methodologies has enabled substantially more detailed analyses of human genome mutations; for example how an increasing rate of *de novo* mutation associated with increasing paternal age, can give rise to disease in offspring.

The Committee considered a paper outlining methods for investigating germ cell mutagenesis, the germ cell genome, meiosis and mutagenesis, the paternal age effect and aneuploidy in germ cells with view to stimulating discussion on chemically induced germ cell mutagenesis. Members noted that it was not known whether unique germ cell mutagens exist (i.e. chemicals that are germ cell mutagens but not somatic cell mutagens). This was mainly due to underutilisation of the currently accepted tests for assessing germ cell mutagenicity and accordingly, a lack of investigations examining the possibility. Differences between mitosis and meiosis meant that it was possible that some chemicals may only be mutagenic in germ cells and this raises some uncertainty over the relevance of somatic cell test endpoints to germ cells.

Members noted that DNA damage in germ cells can be associated with spontaneous abortions, infertility or heritable damage in the offspring/subsequent generations. Methotrexate was given as an example of a pharmaceutical, which can cause teratogenicity through a genotoxic mode of action. It was suggested that exploration of other aspects of the literature (e.g. literature on assisted fertilisation and 3D tissue models for spermatogenesis), may provide some insight into this topic.

A recent paper had identified air pollution as a human germ cell mutagen. It was suggested that this could be explored further although it was noted that measurement of exposure to air pollution can be complex.

A HPA report (prepared by a sub-group of the Advisory Group on ionising Radiation) on transgenerational effects in human populations exposed to radiation (primarily as a result of radiotherapy) was considered as a possible protocol/proof of principle that could be adopted to address chemical induced germ cell mutagenesis. It considered transgenerational effects that were not due to the inheritance of a conventional DNA mutation or mutations arising in the next generation due to the transmission of damaged DNA through sperm (e.g. epigenetic effects). Members acknowledged that there is some evidence for transgenerational effects in mice, but limited evidence in humans. This could be due to humans being less susceptible to such effects or due to transgenerational effects being limited to relatively short times post exposure (i.e. resultant changes or DNA damage may be repaired before conception). There could be species differences in responses to germ cell effects. It was noted that, if embryos with significant chromosome damage were mainly aborted, as may be the case in humans, then adverse effects may occur mainly in terms of impaired fertility or early pregnancy loss rather than as adverse effects in offspring.

Overall, the committee agreed that this would be an interesting topic to investigate further and that future work could be separated into three key themes: i) test methods to identify hazard to germ cells ii) germ cell mutagenesis and ageing and iii) transgenerational effects.

EVALUATION OF CYCLOASTRAGENOL

Cycloastragenol is a novel compound extracted from the root of *Astralagus membranaceus* and intended for use in food supplements. It is reported in the scientific literature that cycloastragenol increases the activity of the enzyme telomerase and thus reduces the number of critically short telomeres but it does not increase mean telomere length. This finding has been reported both in mouse embryonic fibroblasts *in vitro* and in five different types of tissue in mice supplemented with cycloastragenol, as well as in the lymphocytes of human volunteers supplemented with cycloastragenol.

Following concerns that the available data was not robust enough to demonstrate the safety of cycloastragenol in relation to its carcinogenic potential, cycloastragenol was referred to the COC by the Advisory Committee on Novel Foods and Processes (ACNFP) and subsequently the COM.

Cycloastragenol was submitted to the ACNFP for authorisation as a novel food. The submitted data indicated that cycloastragenol has low oral bioavailability and is metabolised to a number of oxidised and hydroxylated compounds. A number of genotoxicity studies were also submitted that were considered by the manufacturers to be either equivocal or negative. Although some information on carcinogenicity was supplied, no standard carcinogenicity studies were submitted. The ACNFP had noted that there was a suggestion of a non-statistically significant increase in liver cancer incidence in treated mice in a study by Bernardes de Jesus *et al*., 2011 which was cited by the applicant. However, the study was limited by small numbers and a relatively short duration of exposure as well as having a relatively high background rate of tumours. This prevented any clear conclusions being drawn from the study. Given, the available data and the reported effects on telomeres, the ACNFP requested advice from the Committee on the Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC).

The COC concluded that the available data were inadequate to demonstrate a lack of carcinogenic potential and that a bioassay or other comparable further work might be needed, although the precise requirements for such work were uncertain given the reported mechanism of the compound. Although the manufacturers considered that the results of the supplied genotoxicity data were either equivocal or negative, the COC requested the COM's view on whether the conducted genotoxicity studies were appropriate to demonstrate a lack of genotoxic potential. The COC were particularly concerned over the *in vitro* chromosome aberration study, which indicated an increase in aberrations at one dose level. A number of suggestions were made for further work regarding genotoxicity that could be conducted. The COC advised that the COM should be consulted regarding potential genotoxicity.

The COM considered the genotoxicity data that had been submitted and whether the package provided was appropriate given the reported effects of cycloastragenol on telomeres and telomerase

<u>COM conclusions</u>

The genotoxicity data submitted for cycloastragenol gave a valid negative result in the Ames test. The findings of the *in vitro* chromosome aberration test results in V79 Chinese hamster cells were equivocal, but this is likely to be an artefact due to cytotoxicity and did not indicate mutagenic potential in this assay.

The results of the available *in vitro* tests indicated that only one *in vivo* genotoxicity study would be required. The *in vivo* erythrocyte micronucleus test in mice via intraperitoneal administration was negative result; there were no concerns over this negative *in vivo* test result with regard to the design and conduct of the study.

Overall, the genotoxicity conducted on cycloastragenol do not indicate *in vivo* mutagenic potential.

The transformed cells used in the chromosome aberration assay (Chinese hamster V79 cells) are sensitive to genotoxicity and already have disrupted telomeres, making it more likely that chromosome aberrations would be produced. Cycloastragenol produced a negative result in this assay, but a chromosome aberration test conducted in non-transformed human cells may have been more relevant biologically.

The potential concern regarding cycloastragenol does not arise from the genotoxicity test results, but from the suggestion of an increase in liver tumours. Therefore, the mode of action in the

target tissue could be investigated further. This could be done by an *in vivo* Comet or micronucleus study in the liver to investigate a possible genotoxic mode of action or by studies investigating evidence for a non-genotoxic mode of action for liver tumours.

The COM also suggested that an expert on telomeres be consulted. Subsequently, Professor Newbold, an expert in telomere biology, was consulted. Professor Newbold was concerned over cycloastragenol because it could remove a key block on the progression of damaged/ageing cells to malignant cells. Professor Newbold also noted that rodent carcinogenicity bioassays would not be biologically representative of humans due to key differences between rodent and human telomere biology.

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. [A formal horizon scanning exercise was not carried out in 2014]. Members considered brief outlines of topics recently reviewed (cell transformation assays; mutation spectra); topics still under consideration and topics proposed for consideration (e.g. gene expression profiling; integration of *in vivo* genotoxicity assays in repeat dose toxicity testing; quantification of genotoxic response; epigenetics and mutations; and 3D tissue models).

Members noted that integrating genotoxicity testing into repeat dose studies was increasingly becoming standard practice (e.g. including MN and comet evaluations in toxicity tests) in order to comply with the 3Rs principles and therefore a review was not considered urgent. It was noted that a paper would soon be published on quantitative approaches to genotoxicity which would inform the Committee on this topic. Members were aware of studies investigating mitochondrial DNA mutations; much of the information was on the impact of mutations on health effects (e.g. neurodegenerative diseases, heart conditions, epilepsy and diabetes etc.) and there was only limited data on associations between environmental chemical exposure and mitochondrial mutations. The Committee agreed to keep a watching brief on this topic. Age related germ cell mutagenesis and its impact on disease in off-spring were also considered to be topics of interest.

PRESENTATION ON 3D MODELS

Following discussion at the Horizon scanning exercise, a Member offered to update COM on recent developments in 3D models for genotoxicity. 3D models for genotoxicity have mainly been developed for the skin (e.g. 3D reconstructed skin micronucleus assay and the 3D reconstructed skin comet assay). The main drivers for the use of such 3D models was the Cosmetics Directive preventing the use of *in vivo* testing for cosmetics and the 3Rs principle requiring the reduction in the use of animal toxicity testing.

The presentation described recent developments in 3D assays. There are different types of models, ranging from single cell microtissues to multi cell types grown within scaffolds. It is hoped the use of such models will reduce the number of misleading positives and improve the accuracy of predictions. Most of the available data relates to skin models, which have the additional advantage of allowing topical application, more realistic metabolism, and use of a skin barrier. Other endpoints can also be evaluated, such as irritation. The genotoxicity 3D models allow both micronuclei (MN) and comet assessments. There is on-going international validation of 3D MN and comet assays. For the 3D reconstructed micronucleus assay, the first phase of evaluation involved optimisation of the protocol, which has been completed. The second phase involved

inter- and intra-laboratory reproducibility with 5 coded chemicals, which has also been completed. Phase 3, pre-validation, with 38 coded chemicals was underway.

Development and evaluation of the 3D comet genotoxicity test has lagged behind that of the MN 3D test and shows relatively poor sensitivity. It was suggested that in terms of regulatory testing of cosmetics, a tiered approach to genotoxicity testing could be used (including the use of the Ames test, *in vitro* mammalian cells and a 3D skin MN/comet).

Advantages of 3D genotoxicity models include the fact that a greater proportion of MN are produced than in 2D models (which could be due to a greater cytochrome P450 enzyme expression) and that they have the potential to assess nano-material genotoxicity via dermal exposure. Current disadvantages of 3D models are that they are relatively expensive; require expertise to conduct; and are at early stages of development requiring further work and validation.
Members noted that it was likely the 3D MN model would be considered by an IWGT working group in 2017. Overall, the COM noted that progress had been made in the area of 3D genotoxicity models and would follow the developments with interest and provide any comment when necessary.

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

Statement 2015/S1 – Statement on the use of mutation spectra in genetic toxicology.

Statement 2015/S2 - Statement on the mutagenicity of alcohol (ethanol) and its metabolite acetaldehyde: update on information published between 2000 – 2014

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