DRAFT **MUT/2015/09**

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

Annual Report for 2014

Draft

Preface 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. 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.

TO BE COMPLETED

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

Draft

MUTATIONAL SPECTRA

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. From this evaluation and discussion, a statement was generated.

A variety of test systems were evaluated. *In vitro* systems included bacterial, human, rodent and transgenic cell lines. *In vivo* systems identified were primarily transgenic models from which genes were more easily isolated and sequenced (i.e. Muta™ mouse, Big Blue and gpt delta mice). The COM agreed that the main value of MS was in evaluating a chemical's mode of carcinogenic action, and in understanding cancer aetiology, including the types of adducts and mutation involved in cancer.

The COM were of the opinion that the selectable genes in the Ames test Salmonella strains and genes such as the *hprt, gpt and tk* loci, in mammalian cells, whilst good systems for identifying chemically induced mutations, are not suitable for use in identification of MS. This is because mutations in such diagnostic genes in bacteria or cells are identified following selection through growth advantage and hence may not be representative of the overall pattern of mutation. Thus, MS are most appropriately identified in phenotypically neutral genes.

The COM noted that the analysis of p53 across different models was of value in evaluating chemically-induced mutations as it has been shown that mutation patterns are conserved in different test systems and species. Mutations in p53 are seen following exposure to PAH's in animals and these are correlated with those seen in some human cancers, for example in smokers, as detailed in an IARC database.

The human p53 knock-in (Hupki) mouse model was also considered. Important limitations in using *in vitro* systems were noted – this includes DNA damage and mutation are more likely *in vitro* than *in vivo* due to the higher levels of oxygen and the greater potential of oxidative damage and results from cell lines may be unrepresentative of untransformed diploid cells.

The COM advised that currently, there were only a few good examples of mutation spectra that could be associated with certain cancer causative agents. These agents are; UV light, aflatoxin B1, tobacco smoke and aristolochic acid. The MS of aflatoxin B1 is distinct in human liver tumours. It was noted that a number of factors could also affect mutation spectra for aflatoxin and other chemicals, such as the effect of viruses (e.g. hepatitis B for aflatoxin) and the time at which spectra are measured (i.e. mutation spectra may change over time after the initial chemical exposure).

Aristolochic acid was considered to generate an unusual tumour and distinct MS. It was considered to be the best example of a specific chemically induced MS. A distinct MS may also be apparent for smoking related exposures, when the sample or measurement was taken at the right time and in the right tissue.

The COM agreed that the development of 'next generation sequencing' technologies, where the whole genome could be sequenced would provide a substantial amount of new data that could be very useful for evaluating and understanding the role of mutation patterns in cancer development.

The COM considered the use of MS from transgenic animal models in interpreting the significance of a positive *in vitro* genotoxicity result and a negative *in vivo* genotoxicity test results, where a chronic carcinogenicity assay was positive. It was suggested that in such cases, any differences in metabolism and target tissues exposure would be considered.

In conclusion, Members agreed:

- Despite an extensive literature describing studies which examine chemically induced mutation spectra in a wide variety of *in vitro* and *in vivo* systems, there are still very few examples where a specific mutation spectrum clearly establishes a specific genotoxic mode of action for a chemically-induced human tumour. Of those highlighted, there is still some uncertainty surrounding the robustness of the spectrum for AFB1 and liver cancer, and although the relationship between smoking, B[a]P exposure and the induced spectrum is characterised, causality remains unproven Aristolochic acid appears to provide the best example for a diagnostic mutation for a human cancer induced by a chemical.
- Mutation signatures using current, single gene, approaches may, on a case-by-case basis, provide useful mechanistic insight into genotoxic modes of action .
- The embryonic stem cells cultured from the HUPKI mouse do not always reflect human p53 mutation response, but as an *in vitro* model they do appear to offer some advantages over other models.
- Major advances in understanding the processes of mutagenesis and carcinogenesis are anticipated when data from studies using next generation sequencing become available. Data to evaluate whether this is the case are awaited.

The full statement is attached:.....

Comment [K1]: This will be attached / referenced

TOX TRACKER ASSAY

The ToxTracker assay is a new genotoxicity test system comprised of a set of reporter cell lines where 6 identified genes reflecting key pathways have been cloned into mouse embryonic stem cells. The COM reviewed the papers published describing the development of the test system and proof of concept exercises and

Draft

the validation data from the test system published to date. Members were also given a presentation by the director of laboratories responsible for developing the assay

The COM Members agreed that the assay appeared to be an interesting approach to identifying genotoxicants and would be potentially useful in evaluating mode of genotoxic action, although it was noted that the selection of the genes used in the test system could have been chosen on an empirical basis rather than on a mechanistic basis. Chemically induced genotoxicity, could be detected by the induction of Green fluorescent protein (GFP) determined by flow cytometry.

According to the Tox Tracker website the entire system comprising six cell lines would be required for the assay to be of sufficient value. Validation data from only two of the cell lines had been published namely the Bscl2 reporter cell line responding to genotoxins and the Srxn1 reporter cell line, responding to pro-oxidants. The COM was not aware of published validation data for the other cell lines, namely Rtkn, Blvrb, Ddit3 and Btg2. The small number of chemicals tested in the presence of S9; a lack of evaluation of the effects of S9 on the expressed genes; and the unexpected results for methyl methanesulphonate (i.e. did not indicate a predominantly genotoxic response); were all considered to be limitations of the data. Currently, the apparent high sensitivity of the test system indicated on the website could not be verified from the published data.

The COM considered that pro-oxidants have genotoxic potential i.e. if the degree of oxidation is sufficient then genotoxicity may occur. It was noted that processes that lead to oxidative stress generated *in vitro* can be very different to those generated *in vivo* (which may also be attributable to immune driven or inflammatory responses).

At a later date, Dr Giel Hendriks from the University of Leiden, was invited to the COM and gave a presentation on ToxTracker. Dr Hendriks provided further insight into advantages of this genotoxicity assay over standard in vitro test systems and some details of the practical aspects of the assay.

Different types of genotoxicity could be detected by certain reporter or biomarker genes related to certain cellular pathways and related biological damage (e.g. DNA damage detected by Bacl2 and Rtkn; oxidative stress detected by Srxn1 and Blvrb; and protein stress by Ddit3). Thus Toxtracker has the capacity to provide some insight into the mechanism of genotoxicity. For validation of the assay, the developers used the ECVAM suggested library for carcinogens and non-carcinogens and the USA Toxcast library.

Optimum sampling time was considered to be 24 hours after initial exposure, a time point sufficiently long to detect aneugenic activity, considered to be a later event. The cut off point for a positive genotoxicity result was chosen as a 1.5 fold increase in the induction of GFP, which was 5 times the standard deviation. All compounds could be tested in the test system with the addition of S9, which is considered to be the most effective method for the inclusion of metabolic activation. Using specifically designed software , the fold increase in GFP induction was used to determine an overall positive genotoxicity response, calculated at a certain degree of cytotoxicity (50% cytotoxicity was selected as the optimum value).

Draft

The COM considered the role of this assay a in a testing strategy. It was suggested that the current view was that it would be useful as an early screen before *in vivo* testing or as a biomarker assay as it does not directly address one of the three mutagenic endpoints (i.e. aneuploidy). ToxTracker may help with the overall interpretation of a scenario where there was a positive *in vitro* genotoxicity result, considered to be weak or a misleading positive and it may be potentially useful in a genotoxicity testing strategy where *in vivo* testing is not permitted, such as in the testing of cosmetics. ItThe committee considered that an inter-laboratory trial for the use of this assay would be useful, but queried how costly and resource demanding the assay would be to use.

The COM agreed that there was a need to gain a better understanding of what the gene expression changes meant in terms of the mechanism of genotoxicity (i.e. what the genes were doing or reflecting) and that an inter-laboratory trial for the assay would be useful.

REVIEW OF THE MUTAGENICITY OF ALCOHOL

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. This review would provide insight to COC regarding possible mechanisms of cancer causally associated with the consumption of alcoholic drinks.

A systematic review of the literature had been conducted to capture the available evidence on the genotoxic effects of alcohol and acetaldehyde. The COM previously evaluated alcohol in 1995 and 2000. The COM published a statement in 2000. The COM suggested that a number of potential confounding factors may also need to be considered in terms of potential cancer risk e.g. body mass index, type of alcoholic beverage, drugs and diet.

The COM made a number of observations of the available data: The potential importance of polymorphisms in genes involved in the metabolism of alcohol, induction of cytochrome P450 2E1 and it's role in the generation of oxidative metabolites were emphasised. It was noted that there were not many good or informative studies on DNA adduct formation following alcohol exposure and key papers on DNA adduct formation were difficult to compare as there may be differences in the sensitivity of the assays employed. The COM considered the adduct N2-ethyl-deoxyguanosine to be a good biomarker of acetaldehyde exposure, but noted the substantial differences in the way studies were conducted (e.g. sensitivity, duration of exposure, and understanding of background adduct levels).

Regarding studies on micronuclei (MN) formation and alcohol consumption, the COM agreed that studies on alcoholics and drug users were difficult to interpret. It was also noted that MN formation in bi-nucleate cells occurs *ex vivo* and there may be a publication bias towards positive results. The studies on MN and chromosome aberrations in alcohol drinkers showed a mixture of effects with only about a ¼ of the studies reporting negative results. T

Comment [K2]: Check

The COM considered potential modes of genotoxic mechanism in some highlighted papers. Studies performed a cytokinesis-blocked MN assay with kinetochore staining *in vitro* which which showed a dose dependent increase in kinetochore positive MN with ethanol treated cells, but not for MN in acetaldehyde treated cells. A study investigating the mechanism of genotoxicity from sub-chronic ethanol exposure in rats suggested that the genotoxicity (as detected by MN) was due to acetaldehyde induced DNA replication lesions in dividing cells. However further investigation would be required before conclusions could be drawn.

The COM agreed that there were sufficient new data to suggest that mutagenicity following exposure to alcohol and its metabolites was biologically plausible. A new statement updating the the conclusions from 2000 will be generated.

Overall the COM concluded:

Comment [OS3]: These are from the first draft statement – need to consider what we publish as part of the annual report

a. Acetaldehyde remains the metabolite of most concern with respect to the genotoxic effects of alcohol.

b. A number of studies have implicated the formation of acetaldehyde-specific DNA adducts and interstrand DNA crosslinks as upstream events in the mutagenicity of alcohol. However, the poor reliability of data available from studies on the genotoxicity of ethanol and alcoholic beverages (the latter being subject to a number of potentially confounding factors) prevent the Committee from drawing any clear conclusions on the genotoxicity of alcohol per se.

c. Studies investigating polymorphisms in key enzymes involved in ethanol metabolism suggest that the ALDH2-deficient genotype is likely to play a key role in the overall mutagenicity **and** genotoxicity of alcohol, although currently available data are inconsistent for polymorphisms in other enzymes.

d. The existing evidence is insufficient to support the suggestion that MN induced by ethanol occur via an aneugenic mechanism and by acetaldehyde via a clastogenic mechanism.

e. There is currently insufficient evidence to support the proposal that acetaldehyde induces MN via formation of replication-associated DSBs in dividing cells. However, the COM acknowledges the plausibility of this hypothesis.

f. Further research is needed to determine whether the recently identified metabolite of ethanol, ethyl sulfate, contributes to the genotoxicity of ethanol and of alcoholic beverages.

Ongoing work: Alcohol- the role of oxidative damage in it's mode of action

The COM acknowledged that oxidative damage is likely to be a key mechanism of concern in the genotoxicity of alcohol and an overview of the potential role of oxidative damage was considered. This included the generation of reactive oxygen

Draft

species, role of CYP2E1 in alcohol-induced liver damage and studies investigating oxidative damage endpoints following alcohol consumption in humans. Of particular note is the generation of etheno adducts. A section pertaining to this will be included in the updated statement on Alcohol and Mutagenicity.

OECD Test Guidelines Programme

During the year COM commented on several OECD genotoxicity Test Guidelines (TG).

- Draft TG in vitro Syrian hamster embryo (SHE) cell transformation assay
- Draft TG474 Mammalian erythrocyte micronucleus test
- Draft TG475 Mammalian bone marrow chromosomal aberration test
- Draft TG473 in vitro Mammalian chromosome aberration test
- Draft TG487 in vitro Mammalian cell micronucleus test
- Draft TG in vivo Mammalian alkaline comet assay
- Draft TG genotoxicity testing for manufactured nanomaterials

Horizon Scanning

The horizon scanning exercise provides information which can be used by Government Departments/Regulatory Agencies to identify important areas for future work. There was no formal horizon scan exercise in 2014. Aspects from 2013 were taken forward in 2014.

Draft