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MUT/05/23

COMMITTEES ON MUTAGENICITY AND CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT. (COM/COC)

STATEMENT ON JOINT MEETING 19 JUNE 2005

USE OF TARGET ORGAN MUTAGENICITY DATA IN CARCINOGEN RISK ASSESSMENT.

Background

1. The COM and COC undertake routine horizon scanning exercises as part of their annual remit (see appended internet links at the end of this statement). The COM identified the use of *in-vivo* target organ mutagenicity studies as a subject for further consideration. The COM and COC agreed to hold a joint meeting on the use of data derived from *in-vivo* target organ mutagenicity studies in carcinogen risk assessment. An open meeting of the committees was held on the 9 June 2005. Attendees included a number of external experts who contributed presentations and comments to the committees discussions. This brief statement has been drafted to record the main outcomes of the meeting. A full write up is being prepared for publication in a peer review publication.

Introduction to current review

2. The interpretation and consequent risk assessment of rodent carcinogenicity data is currently aided by the evaluation of a battery of mutagenicity data.¹ However there are examples of compounds for which equivocal data, or lack of concordance between mutagenicity and carcinogenicity data make it difficult to establish the real carcinogenic risk. This is of particular importance when the organ in which tumours are found is not one of those assessed during *in vivo* genotoxicity tests. Recently the development of methodologies has facilitated the identification of target organ mutagenicity thus offering the potential to more closely define whether tumours seen are attributable to specific mutagenic events.²

Muta[®] mouse and Big Blue transgenic rodent assay systems.

3. An increasing number of rodent carcinogens are being investigated using the Big Blue or Muta[™] Mouse transgenic systems. (For example the COM and COC have recently evaluated transgenic mutation assays as part of the evaluation of malachite green and leucomalachite green.² An important contribution of these assays is that any tissue can be removed and evaluated for the presence of mutations following the administration of a chemical by any exposure route. By demonstrating carcinogen target organ mutagenesis, it can then be inferred that conditions are favourable for DNA reactivity therein

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(e.g. the occurrence of site-specific metabolism).. Site of contact mutagenesis can also be readily studied.⁴ Additionally, sequencing of both the *lac* genes and *cII* is now commonplace and this provides information on the more precise nature of the induced mutations. It is considered that these analyses will contribute to the understanding of target organ tumourigenesis and subsequent risk assessment.⁵ There are several examples in the published literature of how data from Big Blue or Muta™ Mouse have aided carcinogen risk assessment.⁶⁻⁸

The Comet assay

4. The comet assay is now well established as a supplementary assay to the standard battery of genotoxicity tests and is frequently used to evaluate equivocal results from other tests or to investigate the site specificity of tumourigenic responses.^{9,10} Guidelines and recommendations for performing the assay have recently been developed.¹¹ The principle concerns that may arise in respect of the use of the comet assay are; the relevance of the measured endpoint, the robustness and sensitivity of the methodology, especially when assessing chemicals in a range of different cell types.

DNA binding approaches

5. The COM considered the measurement of DNA binding by a number of methods (e.g. postlabelling, radioimmunochemical and HPLC/mass spectrometry) in 1996.¹² The COM agreed that these methods could provide useful data on exposure and uptake to DNA reactive chemicals and metabolites. More recently the COC has considered the application of Accelerator Mass Spectrometry (AMS) in the detection of DNA binding in biological samples.¹³ AMS is the most sensitive technique for measuring the formation of adducts with DNA. AMS technology allows the accurate measurement of very low levels of radiolabelled chemicals (particularly ¹⁴C) in biological samples at around 10⁻²¹ to 10⁻¹⁸ mole. The COC considered that one potential application of AMS was in potential hazard identification. AMS has been recently provided evidence for a lack of DNA binding of 2-phenylphenol and its metabolites in rat bladder which has been important information in concluding a non-genotoxic mechanism for the carcinogenic effect of 2-phenylphenol and its sodium salt in rat bladder.¹⁴

Overview of joint COM/COC meeting on target organ mutagenicity studies.

6. The day's symposium was organised by the Department of Health Committees on Carcinogenicity (COC) and Mutagenicity (COM) with the overall aims of assessing the usefulness of the currently available assays, to provide recommendations on how regulatory bodies should use and interpret these data, and in what situations data should be provided to aid cancer risk assessment.¹⁵ The symposium, attended by Committee members, relevant officials from government agencies and delegates from industry and

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academia, took the form of introductory presentations followed by round-table discussion groups.

Overview of presentations

7. Professor John Heddle (York University, Toronto, Canada) gave the first presentation and provided a comprehensive overview of the usefulness of the transgenic mouse and rat mutation assays in carcinogenicity risk assessment. His principal observations were that the transgenes, *lac I* or *lac Z*, are neutral genes which accumulate mutations linearly over time, ensuring consistency in response which has allowed for the development of optimum protocols, notably the establishment of expression periods.

8. Professor David Phillips (Institute of Cancer Research, Sutton) gave a brief synopsis on the use of DNA adduct detection methodologies in providing evidence of genotoxic mechanisms of action. The techniques are constantly being improved, but it is recognized that the presence of adducts in a particular tissue does not necessarily correlate with tumour induction and are therefore more suitable for addressing mechanistic queries rather than identifying heritable mutagenic effects.

9. Dr Brian Burlinson (HLS) summarized recent developments in the use of the COMET assay, which is rapidly gaining in popularity as a second *in vivo* assay, partially for the reason that the target organ in question can itself be assessed for DNA damage. Importantly OECD guidelines are currently being considered.

10. Dr Phil Carthew (Unilever) presented the pathologists view of the usefulness of target organ mutagenicity data. He pointed out that lack of concordance between mutation frequency, DNA adducts and tumour burden indicate a need to understand more definitively the importance of other steps in the carcinogenic process. However, it is anticipated that data from transgenic mutation assays will be able to provide answers to critical questions, principally with view to hypothesis testing and mode of action evaluation.

Group discussions

Delegates then split into two groups and the ensuing discussions focused around series' of posed questions. Group 1 considered methodological developments, robustness, sensitivity and target organ specificity of the assays under scrutiny, whilst Group 2 addressed specifically the use of target organ mutagenicity data in carcinogenicity risk assessment through questions such as 'how can these data be used to understand the etiology/pathogenesis of rodent tumours?' and 'are these data likely to be more useful for the evaluation of some tumours types and/or target organs?'

Conclusions from Group discussions

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Group 1 (methods) concluded that the transgenic mutation assays were sufficiently robust for general use and that moves should be made to optimise assay conditions and develop protocol guidelines. There were some concerns ultimately regards overall sensitivity and this issue may be heightened when using unusual tissues or chemicals of which little is known (e.g. nature of DNA damage, ADME profile). Nevertheless, it was felt that the limitations of the assay were fairly well understood and this added to a general feeling of confidence in its use. However, it is recognised that the expense of conducting the transgenic assays means that it is likely that the comet assay will be used more frequently, even though they are more suited to detecting clastogens and potentially less sensitive at identifying point mutagens. The comet assay was considered to be satisfactorily validated in most tissues. DNA adducts were not considered useful in a risk assessment scenario, and more likely to be of value for weight of evidence approaches to understanding carcinogenic mechanisms.

Group 2(risk assessment) concluded that the *in-vivo* target organ mutagenesis/ genotoxicity assays provide important information for the mode-of action evaluations for rodent cancer target organs and thus contribute to carcinogen risk assessment. It was considered important to ensure adequate conduct of studies particularly with regard to information on target cell exposure and potential modes of action. Positive results which are not due to high dose cytotoxicity, inflammation or reactive oxygen DNA damage support a non-threshold genotoxic mode of action and that negative results need to be interpreted with regard to the sensitivity of the study used. Clearly, concordant results for several *in-vivo* approaches increase the confidence of conclusions reached.

Overall conclusion

The COM and COC agreed that data from adequately conducted *in-vivo* carcinogen target organ mutagenicity and genotoxicity studies (which included information from investigations using transgenic animals, the comet assay, and approaches to measuring DNA binding (e.g postlabelling and radiolabel methods)) can provide valuable information of use in the mode-of action of carcinogenic responses seen in rodents. Such studies can provide supporting information for use by regulatory authorities in carcinogen risk assessment on a case-by-case basis.

September 2006

(COM/ COC horizon scanning papers for 2004.

<http://www.advisorybodies.doh.gov.uk/pdfs/MUT0422.pdf>

<http://www.advisorybodies.doh.gov.uk/pdfs/cc0432.pdf>)

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