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DRAFT

MUT/04/22

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

HORIZON SCANNING PAPER 2004

1. Members will wish to consider the appended paper prepared by the DH Toxicology Unit and Secretariat which has been focussed on a number of potential topics (i.e. target organ mutagenesis and carcinogenicity in risk assessment, low dose DNA adducts and mutagenicity of micronized chemicals) which might be taken forward in the future. It is suggested that the topic of target organ mutagenesis and carcinogenesis in risk assessment could be taken forward as a joint COM/COC meeting. Recent consideration of chloropropanols and malachite green/leucomalachite green indicate the need for COM/COC to further consider this topic

2. The COM considered a number of topics for potential future consideration during the 2003 horizon scanning process. It had only been possible to take some of these forward during 2003/4 whilst other have been considered by other groups. The potential for formation of mutagens during food processing/cooking (particularly frying) has been taken forward on international fora (e.g The European Food Standards Agency work on acryl amide). Other possible topics included the significance of mitochondrial mutation and the possible genotoxicity of phytoestrogens could be possible topics for future COM consideration, (A COT working group has completed a full review of phytoestrogens). Regarding new methods of evaluation, members suggested there could be a review of changes in gene expression induced by chemical mutagens. (This has been considered as part of the review of Toxicogenomics). It was also suggested that further consideration could be given to the appropriate weight of evidence provided by negative *in-vivo* results in two tissues (as suggested by the COM guidance) when discounting positive results *in-vitro*.

3. Members are asked to consider the appended paper and to provide comments on the priorities for future work.

Secretariat

September 2004

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Committee on Mutagenicity :

Horizon Scanning Exercise 2004

The Committee on Mutagenicity undertakes Horizon scanning exercises at regular intervals. Its function is to identify new and emerging issues which have the potential to impact on public health and which require the Committees' advice. This overview paper has been prepared by the DH Toxicology Unit to aid discussion of identified topics. It should not be considered to be an exhaustive literature review of these subjects.

1) Target organ mutagenesis in identifying cancer risk

The interpretation and consequent risk assessment of rodent carcinogenicity data is currently aided by the evaluation of a battery of mutagenicity and clastogenicity assay data. However there are numerous 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 a variety 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.

The usefulness of Mutamouse and Big Blue transgenic rodent assay systems.

An increasing number of rodent carcinogens are being investigated using the Big Blue or MutaTM Mouse transgenic systems. Its main advantage 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 target organ mutagenesis, it can then be inferred that conditions are favourable for DNA reactivity therein (e.g. the occurrence of site-specific metabolism). Site of contact mutagenesis can also be readily studied.

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.

A review of a large number of published data sets has recently been performed by the IPCS; the final publication is awaited.

There are many examples of how data from Big Blue or Mutamouse has aided risk assessment decisions regards potential mutagenicity; for example

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Suzuki et al (1998, 2000) used MM to look at target organ mutagenesis of quinoline which produces liver tumours in rodents but is negative/equivocal in a liver UDS assay. Positive transgenic assay results were obtained and this was shown to be associated with an increase G:C to C:G transversions. This provides evidence strengthening the case for a genotoxic mechanism of this compound. Von Pressentin et al (1999) demonstrated the utility of Mutamouse to identify site of contact mutagens by looking at mutations in the tongue. Turner et al (2001) investigated the rodent carcinogens d-Limonene and sodium saccharin using the Big Blue rat; both compounds failed to increase mutation frequency in target organs thus substantiating their non-genotoxic mechanisms of action.

The usefulness of the Comet assay

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 (for example, Hartmann et al 2004, Sekihashi et al 2002). Guidelines and recommendations for performing the assay have recently been developed (Hartmann et al 2003) and UKEMS are currently involved with the OECD harmonization process.

The principle queries that arise 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.

The usefulness of mutation data spectra and microarray analysis:

It is recognized that the CoM has recently reviewed in great detail the role of the genomic type methodologies and their value in assessing genotoxicity. However because of the constant and rapid development of these techniques, it is suggested that this topic should be further considered. By establishing a data base of microarray profiles/fingerprints of known carcinogens/those with understood mechanisms of action it is hoped that this will aid the elucidation of the mechanism of action of those with unknown mechanisms. It is apparent that the actual functional significance of each up/down regulated gene is not always useful; it is the fingerprint which is expected to be the useful.

Recent data include:

Hu et al (2004) aimed to identify gene expression profiles that discriminates indirect acting genotoxins from direct acting genotoxins. The authors looked at DNA reactive agents of different mechanisms (MMC, MMS) and those that act via other mechanisms (e.g. topoisomerase inhibitor, microtubule inhibitor) with view to establishing distinct fingerprints for each mechanism of action.

Examples of compounds where overall generic guidance would be of value in the risk assessment process include:

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Malachite Green and Leucomalachite Green

MG: Mutagenicity tests provided inconsistent *in vitro* positives, borderline *in vivo* positives plus adducts and equivocal results in the rat liver following 2 year bioassay, with possible indication of epigenetic mechanism. LMG: Negative data in *in vitro* assays and *in vivo* BMMN, but low level DNA adducts in rodent liver. Carcinogenicity data from NTP is equivocal, with the only clear evidence of carcinogenicity noted in the liver in female mice (adenomas) fed LMG. Recent published data suggests an increase in mutation frequency in the Big Blue mouse liver following administration of LG, but not MG, and not in Big Blue rats (Mittelstaedt et al 2004). Additionally, the mutation spectra in treated animals was different from that seen in controls (increase in G→T and A→T transversions). The interpretation of the significance of the DNA adduct data and mutagenicity data with regard to the mechanism of carcinogenicity of LMG is complicated by the paucity of the evidence for carcinogenicity. How does this data contribute to the risk assessment?

Daminozide

This has been used as a pesticide on fruit crops in the US, but the borderline mutagenicity data means it is now used only as a plant growth regulator. Genotoxicity data is inconsistent (equivocal *in vitro* data, negative BMMN and UDS, positive comet), (http://www.efsa.eu.int/science/ppr/ppr_opinions/453_en.html), whilst bioassays (NTP) in rats and mice showed increases in some tumour incidences (most clear evidence being in female rats where adenocarcinoma of the endometrium and leiomyomas in the uterus were seen, but also hepatocellular carcinomas in male mice). In this instance the carcinogenicity risk assessment is complicated by the inadequacies/inconsistencies in the mutagenicity data. Would target organ mutagenicity data improve the risk assessment?

Alachlor a chloroacetanilide herbicide

Nasal, thyroid and stomach tumours are seen in rats following oral administration, but no tumour increases are seen in mice. The genotoxicity data equivocal. Heydens et al (1999) interpretation of the available data suggests that the mechanism is non-genotoxic, based on metabolite induced increased cell turnover in affected tissues or via thyroid pituitary axis (via increased synthesis of TSH). Interestingly, array profiling has been used to evaluate a time course of gene expression changes in olfactory mucosa (Genter et al 2002), although these data provide more information on the tumour progression process rather than the presence of absence of an initial mutagenic event. This is another good example of where tissue-specific mutagenicity data would aid the risk assessment.

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Questions for COM:

What is the true value of new methodologies in characterising and quantifying risk?

How can these data be extrapolated to enable more accurate risk assessments?

What are the caveats in using these data?

Is it possible to draw comparisons between mutation spectra from transgenes with target organs from carcinogenicity studies?

It is suggested that this topic could be developed for a joint meeting of the COM/COC.

2) Risk assessment using low dose DNA adducts

Techniques to assess DNA adducts are constantly being improved and with the increasing use of highly sensitive methodologies, such as accelerator mass spectroscopy and atomic force microscopy, the level of detection of DNA adducts is fast approaching 1×10^9 . However the use of these data in carcinogen risk assessment is not commonplace due to the underlying difficulties in interpretation such as the conversion of DNA adducts to mutation, the role of specific mutations in the carcinogenic process, and influence of repair processes. An ILSI/HESI meeting in April 2004, aimed to generate guidance on the use of these techniques in risk assessment.

Aflatoxin is the most widely quoted example of where DNA and albumin adducts have been used to assess carcinogenic risk in man, as tumour incidences have been quite accurately predicted from molecular dosimetry of adduct data (Wang et al 2001). There is also a large body of data obtained from the evaluation of the carcinogens PhiP and MeIQ. For example, Muathe et al (1999) showed that there are a greater number of MeIQ adducts formed in human colon than in rodent colon. What are the implications of this? Are humans more susceptible?

Fukushima et al (2002) demonstrated DNA adducts following administration of N-nitroso compounds at dose levels which did not cause tumours in the same model. Does data such as this provide evidence of a threshold mechanism and what further information would be required to extrapolate this to a human risk assessment.

Examples of other areas of recent attention and development are, : differing persistence of adducts in different tissues can be explained by the role of DNA repair in different tissues. What is the relevance of this observation on the use of the technique in risk assessment?

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The use of DNA and biomarkers adducts as biomarkers of exposure is reviewed by Farmer (2004). How should this development be used in the assessment of risk? DNA and protein adducts as markers of genotoxicity

Questions for COM

What is the relationship between measured adduct levels, exposure and dose?

What is the value of low dose DNA adduct data and how can it be used in carcinogen risk assessment?

Do low levels of DNA adducts represent a hazard to health?

What methodological considerations (controls, artefact identification) need to be taken into account?

[The COC/COM held a joint meeting on the significance of low level DNA adduct data in carcinogen risk assessment in 1996. Overall the symposium concluded that DNA adducts were a useful indicator of exposure and uptake but couldn't be used in the risk assessment of carcinogens].

3) The genetic toxicity of nanotechnology products and nanoparticles.

Nanotechnology is defined as engineering and medicine on a nanometre scale and involves manipulation and construction at molecular and atomic levels. It's usefulness in biological and medical fields is expected to be broad; of particular interest for the current deliberation are, nanoparticulate drug delivery systems, the use of nanoparticles in cosmetics and potentially the construction and use of nanotubes.

The Royal Society has recently published it's report in which the potential environmental, health and safety implications of nanotechnology were addressed. <http://www.royalsoc.ac.uk/nanotechnology/> and www.nanotec.org.uk

Toxicological concerns centre mainly around differences in the toxic potential of nano-particles, consequences of increased uptake or increased reactivity. In particular, attention has focused on air pollution; observations have been made where toxic responses by apparently non-toxic substances occur when inhaled in nanoparticulate form (Donaldson et al 2002). Mechanisms of toxicity may include the generation of reactive oxygen species following macrophage engulfment

Currently, there is little data on the genetic toxicity of nanoparticles. Micronized zinc oxide (0.2µm) has been shown to be more clastogenic than unm micronized chemical (SCCNFP/0649/03). Similarly, Rahman et al (2002) provides evidence that ultrafine titanium dioxide, a normally inert compound, induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. It is

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postulated that nanoparticulates stimulate phagocytosis, thereby generate the production of reactive oxygen species.

Are there any toxicological considerations needed for the use of nanoparticles, in particular given that one intended use is in cosmetics?

Nanotubes, are also discussed in the Royal Society paper. These are described as resembling rolled up graphite sheets, with dimensions 1nm wide, and up to several mm long, and are capable of conducting electricity. A few studies have demonstrated the toxicity of nanotubes; for example Lam et al (2004) reported inflammation in the lungs following intra-tracheal administration, whereas equivalent quantities of carbon black did not.

To date, there are no reported investigations of the potential genetic toxicity of nanotubes. It is well established that the disturbance of the mitotic spindle by fibres with highly specific dimensions, such as asbestos, is critical to its carcinogenic potential. Could similar mechanisms present a genotoxic potential for nanotubes?

An example of a nanoparticle formulation designed to increase the local concentration of a drug. It was demonstrated that nanoparticles were rapidly and more extensively internalised into cultured cells.

Questions for COM

Do these data raise questions regarding the design of mutagenicity test strategies for nanoparticulate chemicals or other nanotechnology products ?

4) The role of micronutrients in preventing mutagenesis

There is considerable attention being paid to the role of micronutrients in the prevention of cancer. Topics of particular interest in the current paper include the following:

Nutrigenomics: defined as the interaction of nutrition and an individual genome. It is postulated that the variation in cancer incidences within populations with similar dietary patterns may be explained by genetic factors (Davis and Miller 2004). For example, this may be consequence of polymorphisms of nutrient metabolism.

Methylation status: it is considered that diets lacking micronutrients which are known to contribute methyl groups can result in altered DNA methylation status (Ross and Poirier 2002; Bombail et al 2004). This in turn is likely to impact on mutation /cancer susceptibility

Antioxidants: for example the vitamins C&E, selenium and lycopene are considered to impact on *in vivo* mutagenesis.

An example of investigation of this protective potential has been described by De Boer (2001) who used Mutamouse to look at the modulation of mutagenic potency of known carcinogens by dietary compounds.

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Advice on the nutritional implications of the research reviewed above is the responsibility of SACN (Scientific Advisory Committee on Nutrition). It is noted that some of the developing scientific methods would impact on COM expertise. It is suggested that the SACN secretariat is made aware of COM views.

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