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MUT/05/21

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

HORIZON SCANNING PAPER 2005

1. Members will wish to consider horizon scanning topics identified by the secretariat and DH Toxicology Unit Secretariat. A limited literature search was undertaken using PUBMED which indicated over a thousand publications in 2004/5 on chemical related mutagenicity and related topics which might potentially be relevant. The literature search was scanned briefly to highlight potential chemicals and generic areas of mutagenicity evaluation which might be of interest to members. A brief overview has been produced below. The horizon scanning exercise also provides an opportunity for members and advisers from Government Departments/Regulatory agencies to suggest topics for further work.

Specific chemicals identified in literature search

A report of a positive dermal bone-marrow micronucleus test with paraquat was identified (D'Souza UJ et al Mut Res, 581 (1-2), 187-190, 2005). This was an unexpected result and has been referred to PSD. [There is evidence that paraquat may induce micronuclei by an oxidant pathway, (Ortiz GG et al Mut Res, 464 92), 239-245, 2000). However a positive response following dermal application was unexpected given the view that paraquat is absorbed to a very limited extent following dermal application to rats (Chui YC et al Tox Ind health, 4 (2), 203-219, 1988)]. The COM would be consulted if a need for specialist advice was identified.

The Committee identified the potential for mitochondrial DNA as a target for mutagenic compounds during the 2003 horizon scanning exercise. Quite recently Liu SX and colleagues (Cancer Research, 65 (8), 3236-3242, 2005) have published an *in-vitro* study of sodium arsenite using A_L (human-CHO hybrid cells) using loss of expression of CD59 surface antigen in mutant cells as an assay for mutagenicity. Studies were undertaken in enucleated or mitochondrial DNA deficient cells. Arsenite treatment of enucleated cells resulted in significant mutagenicity whereas treatment of mitochondrial depleted cells resulted in few or no mutations. Arsenite induced mitochondrial damage was linked with the formation of superoxide and peroxynitrites. This is one of the first demonstrations of mitochondrial DNA induced mutagenesis submitted to COM. These data do not change the risk assessment approach for arsenic compounds which is based on the conclusion that trivalent arsenic compounds are genotoxic carcinogens. The COM is asked to consider what potential role mitochondrial DNA might have with regard to mutagenesis? Is this a potential area where testing strategies may be deficient?

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The dinitropyrene isomers are known to be highly potent *in-vitro* mutagens in *Salmonella typhimurium* strains. (see WHO IPCS Environmental Health Criteria document 229, 2003) NitroPAHs originate from PAHs (generally adsorbed on particulate matter and themselves products of incomplete combustion). The octanol/water partition coefficients for nitroPAHs are high, indicating that nitroPAHs adsorb strongly to the organic fraction of soils and sediments. Leaching into groundwater is therefore thought to be negligible. Very recently Watanabe T and colleagues from the Department of Public Health, Kyoto Pharmaceutical University have reported in a brief communication, the isolation and *in-vitro* mutagenicity testing of 3,6-dinitrobenzo(e)pyrene in soil samples from three metropolitan areas in Japan. This is the first published report of this compound being identified in soils. It was reported that 3,6-DNBeP induced 285,000 revertants/nmol in TA98 and 955,000 revertants/nmol in YG1024 without S-9 mix. These data would suggest that 3,6-DNBeP has similar *in-vitro* mutagenic potency to 1,3-1,6, and 1,8-nitropyrenes. (Watanabe T et al, Chem Res Toxicol, 18, (2), 283-9, 2005.)

Mutagenicity evaluation and testing of mixtures

It is widely recognised that we are exposed to complex mixtures of chemicals environmentally, occupationally, therapeutically or via a combination of these. However toxicology testing strategies (including genotoxicity/mutagenicity) are largely based upon the evaluation of single chemicals. The risk assessment of chemical mixtures is characterised by models in which chemicals within a mixture are considered to fit either dose additive, response additive or interactive profiles (Jonker et al 2004). In general it is believed that genotoxic chemicals fit the dose additive model whereby they are assumed to behave similarly in terms of mode of action and combined responses can be calculated from dose responses of the constituents of the mixture. 'Bottom-up' investigative approaches, where knowledge of the toxicities of individual chemicals are modelled to give an overall assessment of risk, can be performed when the mixture is simple (few components, well defined) and/or when sufficient data is available (examples; a pesticide product). 'Top-down' approaches are used for complex mixtures (many components, ill defined) and involve testing the whole mixture and comparing results to those achieved with individual components. Examples of complex mixtures include petroleum hydrocarbons, contaminated land/waste sites and drinking water.

For genotoxic carcinogens in the environment, the requirement is to maintain levels as low as is reasonably practicable (ALARP). With this in mind, there are two potential issues which may arise when considering the mutagenicity/genotoxicity of a mixture:

1) Is the assumption that all genotoxins will adhere to the dose additive model correct? (Expressed mathematically as : $E_{AB}(d_A, d_B) = E_A(d_A) + E_B(d_B)$: where A and B are two chemicals in the mixture and E and d represent the effects and dose of individual compounds respectively), or are there scenarios

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that interactions (either synergistic or antagonistic) will occur, when the presence of a first mutagen affects the potency of a second?

Said et al (1999) demonstrated the enhancement of the bacterial mutagenicity induced by N-acetoxy-acetylaminofluorene by pre-treatment with AFB1-8,9-epoxide and suggested that pre-existing adducts may distort DNA thus leading to potentiation of intercalating agent effects. It is also suggested that the ability of arsenic to modulate DNA repair *in vitro* enhances genotoxicity in CHO cells (Lee-Chen 1993, Wiencke and Yager 1992) and similar conclusions have been reached regarding the potential mechanism of cobalt induced genotoxicity (De Boeck et al 1998).

2) If a complex mixture is tested and found to be positive *in-vitro* or *in-vivo*, should the mixture simply be regarded as a mutagen or should efforts be made to tease out the active components. For example, in a study assessing the mutagenicity of settled house dust, it was demonstrated that only 25% of the mutagenicity could be accounted for by known PAHs (Maertens et al 2004). Furthermore, how should the variability of complex mixtures such as drinking water be accounted for when assessing the risk?

This concern is borne out by the analyses of available data presented in the WiGRAMP report on risk assessment of chemical mixtures. Weaknesses were revealed in the design of many studies which had attempted to assess the genotoxicity of mixtures, as it was not possible to draw conclusions on potential interactions (e.g. individual compounds not tested separately, inadequate dose responses).

Do the Committee think further assessment of the potential interactions between genotoxic chemicals is warranted?

Should specific genotoxicity testing strategies be developed to aid those involved in the risk assessment of chemical mixtures?

Potency indicators for mutagens

Members will recall that Sanner and Dybing developed the carcinogen potency indicator T_{25} which has been useful in developing crude carcinogen ranking approaches. In a recent paper, the same authors have sought to develop a genotoxicity potency estimate which could be used for prioritisation of mutagens in situations where there are no appropriate carcinogenicity data. The LED (Lowest Effective Dose) can be best described as a genotoxicity potency indicator as it is based on *in-vivo* data derived from rodent studies using a wide diversity of end points including DNA adducts, micronuclei, DNA breaks, chromosome aberrations, and SCEs. The authors excluded data from host-mediated assays, RNA adducts, and protein adducts. A search of the IARC monographs database was undertaken to identify chemicals where oral/or inhalation T_{25} and LED could be derived. From an initial list of 44 carcinogens, those where there was evidence for a non-genotoxic MOA were excluded resulting in an analysis of the ratio of LED/ T_{25} (lowest value

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selected) for 34 carcinogens. The authors reported a linear correlation. The median ratio of LED/T₂₅ was 1.05 with 90% of substances in the ratio 0.21-0.9. The authors suggested the numerical value of LED was similar to T₂₅ within a factor of 5-10. What are members views of this proposed approach? (Sanner T and Dybing E, Basic & Clinical Pharmacology and Toxicology, 96, 131-139, 2005). The authors also report a further new study comparing animal carcinogenicity potency with information on carcinogenic potency from epidemiological studies.

Members are asked whether it would be valuable to review this work in more detail.

Developing a rationale for further consideration of mutagenicity test strategies

Sensitivities: UDS versus COMET

The current COM mutagenicity test strategy utilises the rat liver UDS assay as the second *in vivo* assay if required. However, since the implementation of these guidance (2000) the COMET assay has been extensively developed and validated and has the added advantage of using a variety of different tissues. At the recent Target Organ Mutagenicity Symposium (9 June 2005 , <http://www.advisorybodies.doh.gov.uk/pdfs/Prog090605.pdf>) it was suggested that the general utility of the UDS assay is waning, due to potential sensitivity issues and the inflexibility of only using a single tissue, whilst the COMET assay is gaining in popularity as a second *in vivo* assay.

Do the Committee think it would be useful to conduct an evaluation of the relative sensitivities of the *in vivo* COMET assay compared to the liver UDS assay? If so, would data available in the public domain suffice or would we need access to anonymous development compound data?

Validation of test methods

Members briefly discussed the developments in the approaches to testing for in-vivo mutagenicity during the 9 June 2005 joint meeting with COC on target organ mutagenicity. It is suggested it would be useful for the committee to briefly review the current developments in approaches to mutagenicity testing. In this respect, one suggestion is to hear and discuss a presentation from an expert on the development and application of novel mutagenicity tests Dr David Kirkland has provisionally agreed to undertake this at a suitable meeting. What are members views?

Evaluation of CHO hprt assay

An evaluation of all CHO hprt assays submitted to the ACP which were undertaken in accordance with the 1997 OECD guideline is being undertaken jointly by COM secretariat and PSD. The object is to look at this

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predominantly negative database and to consider aspects of the conduct of the assays which may have influenced the outcome. Of key interest is the number of cells treated, the number of cells undergoing selection treatment, the influence of cytotoxicity on conduct of the assay, the finding of trials with complete set of clear plates, the historical control data, and the mean fold increase reported with positive controls. Particular interest will be taken with regard to studies where there is evidence of a treatment related increase in mutation frequency and the arguments advanced for discounting such an increase. It is hoped the outcome will provide interesting and relevant information on the application of the CHO *hprt* assay from a regulatory database of predominantly negative studies. The studies originate from many laboratories but all were judged to conform to the relevant OECD guideline. The data will be compared with published evaluations of the CHO *hprt* assay. Particular interest will be taken in the EPA validation trials where investigations of the sensitivity of the CHO *hprt* assay to weak mutagens were undertaken. It is hoped a paper will be available during 2006.

Role of methylation status: Transgenerational effects of methylation.

The Medical and Toxicology panel of the ACP reviewed a recent paper which reported on investigations into the potential for vinclozolin or methoxychlor to induce transgenerational effects via the male line following a short duration exposure of pregnant females to relatively high doses. Decreased spermatogenic capacity and reduced fertility were reported over four generations. The authors suggested that the effects on reproduction correlation with altered DNA methylation. The MTP noted there was a considerable literature available on transgenerational effects of chemicals in experimental animals (for example with diethylstilbestrol, e.g. Newbold RR, Toxicol Appl Pharm, 199, 142-150, 2004) and hence it was important to consider the scope of any review work, and which potential epigenetic mechanisms should be considered and also the potential end points under consideration. It was suggested that PSD and DH might consider potential research priorities. In this respect it would be valuable to have advice from COM on the role of DNA methylation and changes in methylation status with regard to the potential for transgenerational effects. One further reference was also noted during the drafting of the horizon scanning paper. Shio YH and colleagues reported on the allele-specific germ cell epimutation in spacer promoter of the 45S ribosomal RNA gene after exposure of mice by intraperitoneal injection to trivalent chromium. The authors also reported DNA methylation pattern changes in sperm DNA. (Shiao YH et al, Toxicol Appl Pharm, 205, 290-296, 2005).

It is possible that any review of this subject would be a considerable undertaking. How would members suggest the work be focused to obtain the best possible advice on research priorities?

Approaches to Biomonitoring

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The COM has completed a large review of biomonitoring studies in pesticide applicators. Relatively few of the studies considered used the COMET assay. Faust F and colleagues reviewed the utility of the comet assay for biomonitoring for exposure to occupational genotoxicants using primary blood cells. (Faust et al Toxicol, 198, 341-350, 2004.) The authors note the ease, practicality and high concordance with cytogenetic assays as positive aspects. The apparent deficiencies in the biomonitoring studies predominantly related to problems seen in the pesticide biomonitoring studies and included small study size, bias in distribution of gender, lack of qualitative and quantitative exposure data, and omission of confounding factors (information on physical activity and diet are noted). Factors related to performance of the Comet assay included lack of uniformity of assay procedures and inconsistency between the Comet assay and other tests for genotoxicity and with results with known mutagens/carcinogens.

Do members agree with these views? Are there any aspects to biomonitoring for exposure to genotoxins which members would wish to consider in the future?

Conclusions

A number of potential areas of mutagenicity evaluation and testing have been highlighted for members consideration. This includes consideration of the potential importance of mitochondrial DNA as a target for mutagens, approaches to the evaluation and testing of mixtures, the use of mutagenicity data as potency indicator for carcinogens, an evaluation of the sensitivity of UDS and COMET assay in mutagenicity screening, a presentation and discussion of validation and use of developing mutagenicity tests, an evaluation of the utility of the CHO hprt assay using a predominantly negative dataset, a review of the role of methylation status with regard to transgenerational effects, and further consideration of genotoxicity as a biomarker of exposure to mutagens.

Members are asked to consider these ideas and to add suggestions. The Committee will be asked to give an indication of priority. The scanning exercise doesn't take account of any reactive work the Committee might be asked to undertake (e.g. a request from a Government Department).

Secretariat/DH Tox Unit August 2005.

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