

MUT/07/10

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

**DEVELOPMENT OF A HIGH THROUGHPUT GENOTOXICITY SCREENING ASSAY USING GREEN SCREEN HC GADD45a-GFP. (Growth Arrest and DNA Damage gene)**

*Hastwell PW et al Mutation Research, 2006 e-publication*

1. Members will wish to note this publication, which the secretariat has provided as additional information to accompany the presentation from Dr R Walmsley (University of Manchester). The publication provides information for GADD45a-GFP genotoxicity assays in TK cells with an Epstein Barr Virus based-plasmid containing human GADD45a operatively linked to GFP for a selected group of 75 chemicals. The secretariat notes a number of topics which members may wish to consider. These include;
2. The evidence base for a wide range of genotoxicant and other stressors resulting in induction of GADD45a in mammalian cells. How specific is the response to genotoxicants?
3. The method used to determine a stress response including selection of doses, use of 24 and 48 h cultures, selection of controls (including absorbance control for culture and also exogenous metabolising fraction). This consideration would also include the influence of background chemical effects on determining absorbance and fluorescence. The high through put using manual (ca 50 compounds/day) and potentially higher through put using automated systems is noted.
4. The criteria and data used to establish GADD45a positive response (50% increases on background) and cytotoxicity (30% RSG) given the use of 150,000 cells per microwell. It is noted dose-response data were presented in the publication for a number of chemicals, with the majority of data reported as LEC values (it is not clear if this is 24h or 48h data or a combination of both times). Dose-response evaluation criteria were not reported.
5. The preliminary data on use of exogenous metabolic activation to measure genotoxicity of cyclophosphamide. Would more chemicals using metabolic activation be required?
6. The evaluation of sensitivity and specificity reported for the GADD45a-GFP assay compared to some mutagenicity assays currently used in regulatory testing schemes. The authors include that the proposed approach using GADDD45a improves both sensitivity and specificity of

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prediction of carcinogens in comparison to the existing in-vitro package of tests (Ames, in vitro CA or MLA, and in-vivo CA or MNT).

*COM discussion*

7. Members will wish to recall that the current COM guidance for the use of three in-vitro mutagenicity tests for the majority of chemicals (in-vitro bacterial (*Salmonella typhimurium* /*E.coli* strains), in-vitro mammalian cell tests for clastogenicity and aneugenicity (chromosomal aberrations, the COM guidance also recognises that the in-vitro MN assay may be used) and in-vitro mammalian cell mutagenicity (MLA preferred). Generally a negative response in all three tests is sufficient to conclude that there is no potential for mutagenicity (except high exposure compounds). A positive in any one of the three in-vitro mutagenicity tests indicates the need for an in-vivo mutagenicity testing.
8. How do members see the GADD45a test within the strategy as a replacement (e.g. for mammalian cell tests) or as a complimentary screen undertaken prior to mutagenicity testing. It is noted that all of the current mutagenicity tests in the COM guidance provide some information on the nature of the effects seen and interaction with DNA and help with selection of appropriate in-vivo testing strategies.

**Secretariat March 2007**