

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)**DRAFT****Thresholds in mutagenicity: some studies published since 2001.**

- 1) This paper is intended to provide some background on the subject of genotoxicity threshold mechanisms to inform prior to the presentation to be given by Dr Gareth Jenkins (University of Swansea).
- 2) The topic of thresholds in mutagenicity has been considered by the COM on various occasions. In the COM statement on risk assessment of *in vivo* mutagens published in June 2001 (COM/01/S3), it was agreed that two mechanisms exist for which a threshold for a genotoxic response occurs; aneugenicity induction by tubulin inhibitors (specifically the methyl benzimidazoles carbendazim and benomyl) and the rapid detoxification of hydroquinone and phenol via the oral route. In the conclusions it was reaffirmed that for *in vivo* mutagens it is prudent to assume that there is no threshold for mutagenicity but when a potential threshold related mechanism has been identified, appropriate data should be generated on a chemical-by-chemical basis.
- 3) The COM considered a paper in February 2004 which looked at genotoxic carcinogens and DNA repair at low doses (MUT/04/14). Here, papers studying a number of DNA repair systems *in vitro* were reviewed for the occurrence of thresholds or 'J' shaped dose response curves. In short, evidence for thresholds in the induction of mutations was found and this was most apparent in the induction of O⁶-methyl transferase (O⁶-MT) in bacterial systems following incubation with ethylating and methylating agents such as EMS, MMS, ENNG and MNNG. Overall, the Committee agreed that there was no clear evidence for a J shaped dose response in any of the data considered. Data regarding the O⁶-MT induction suggested that an *in-vivo* threshold was likely, but not proven. No conclusions could be drawn from the limited data on other DNA repair mechanisms.
- 4) The COM has previously considered a paper by Dr Jenkins 'Do dose response thresholds exist for genotoxic alkylating agents? (Mutagenesis 20 389-398 2005 : MUT/06/17, appended). The Committee concluded that the concept of a threshold for biological significance could be a useful way forward, but felt that this needed to be considered in the context of the possible DNA repair mechanisms involved and the available dose-response data available (including the sensitivity of the method to detect a NOEL) and did not significantly impact on the advice and opinion provided in the 2001 statement. Regarding future work, members agreed with Jenkins et al that further studies with paired alkylating agents with similar/dissimilar adduct types with repair deficient cell lines could be informative. It was agreed that it would be important to monitor future literature in the area of thresholds for *in vivo* mutagens.
- 5) A number of other papers have been identified which provide some evidence for thresholds in mutagenicity. Kaina et al (1998) reviewed the use of transgenic cell lines and

animals and knockouts which consider the role of DNA alkylation damage and the mechanisms by which these lesions are converted to critical genotoxicity endpoints. Notable observations included; mutagen treatment of cells with and without O⁶-methylguanine-DNA methyltransferase (MGMT) showed that O⁶-methylguanine was responsible for cell death and mutagenic/clastogenic responses thus representing a tumour initiating lesion but was also shown to induce apoptosis which MGMT protects against. Assessment of sister chromatid exchange (SCE) as a measure of DNA damage, induced by MMNG in CHO-9 cells expressing and not expressing MGMT showed that MGMT inhibited second cycle induced lesions, but had no effect on first cycle lesions. Furthermore, the dose response curves were clearly not linear suggesting a threshold of response at dose of approx 10µM MMNG. *In vivo*, over-expression of MGMT provided protection against low dose of MNU in a skin painting model, although an absolute threshold was not evident. Other mechanisms were also considered in the paper. Examination of base excision repair activity of N-methylpurine-DNA glycosylase, apurinic endonuclease and DNA polymerase β in transgenic systems generally showed that down regulation was associated with an increased response to mutagenic, DNA binding activity.

6) Hansen et al (2007) examined the role of MGMT in *Mgmt* *+/+* and *Mgmt* *-/-* mice treated with alkylating agents temozolomide, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and cyclophosphamide. It was demonstrated that MGMT significantly protects against *in vivo* TMZ induced Hprt mutations but not CP or BCNU induced mutation. The significance of this in relation to human chemotherapy was discussed.

7) In a paper by Doak (2008) the differences in the linearity between different alkylating agents was examined. It was suggested that this was dictated by the alkylation target within the bases and that there was some evidence with a clear mechanistic basis for the presence of threshold. For alkylating agents: adduct profiles for ENU, EMS, DES, MMS, MNU and MNNG reactivity of an alkylating agent is described by Swain Scott constant (s-value range 0 to 1), high s value target nucleophilic centres such as O⁶ guanine. Those with low s-values are less likely to exhibit a threshold response. Human lymphoblastoid cells (AHH-1) were exposed to MMS or EMS for 1, 2, 4, 6 or 24h after which mass spectrometry for adducts, gene expression for MGMT, n-methylpurine-DNA glycosylase (MPG) and p21 and western blotting for protein expression of these genes was undertaken. N7-meG DNA adducts were detected and concluded that it is high s-value chemicals that induce these predominately. MPG expression was not significantly affected but MGMT was increased and apparently at dose below the *in vitro* NOEL for MMS. For EMS, the expression change was seen only above the NOEL. The authors conclude that there may be differences in the way different adducts are removed, ethyl adducts eliciting a weaker response.

9) Reviewing the literature, it is evident that Dr Jenkins and his team are some of the leaders in this area of research. The role of DNA repair in the generation of threshold mechanisms remains plausible and relevant.

References:

Doak, S.H, Brusehafer, K, et al (2008) No-observed effect levels are associated with up-regulation of MGMT following MMS exposure. *Mut. Res.* **648** 9-14

Hansen, R.J., Nagasubramanian, R., Delaney, S.M. et al (2007) Role of O⁶-methylguanine-DNA methyltransferase in protecting from alkylating agent-induced toxicity and mutations in mice. *Carcinogenesis* **28** 1111-1116

Jenkins, G.J., Doak, S.H., Johnson, G.E et al (2005) Do dose response thresholds exist for genotoxic alkylating agents? *Mutagenesis* **20** 389-398

Kaina, B, Fritz, G, Ochs, K et al (1998) Transgenic systems in studies on genotoxicity of alkylating agents: critical lesions, thresholds and defense mechanisms. *Mut. Res.* **405** 179-191