

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

Second Draft Working Paper on the Genotoxicity of Acrylamide

Additional Literature Search

1. As part of the on-going review of the available evidence of acrylamide genotoxicity, conducted at the request of the Health and Safety Executive and the Food Standards Agency, the Committee is presented an updated systematic literature search which includes references published after the last full search in July 2007.
2. This search identified 45 new references, 35 of which did not meet the inclusion criteria. Of the remaining 10 papers, 5 had already been considered by the Committee, having been either identified by the Secretariat or in Polyelectrolyte Producers Group (PPG) submissions. The remaining 5 references have been summarised below. The Secretariat has copies of these papers, and a copy will be available at the meeting. If Members require a copy of any of these papers, please contact the Administrative Secretary.

Summaries of new references

Mei N, Guo L, Tseng J, Dial SL, Liao W, Manjanatha MG. (2008) Gene expression changes associated with xenobiotic metabolism pathways in mice exposed to acrylamide. Environ Mol Mutagen. [Epub ahead of print]

Acrylamide (500 mg/L in drinking water for 3 weeks) was administered to male mice. Although species was not stated, this appears to be additional work using samples from the Big Blue transgenic mouse work by Manjanatha *et al.* (2006). RNA was extracted from liver samples from 6 control and 4 treated mice. Each sample was examined in triplicate by RNA 6000 LabChip and Agilent 2100 Bioanalyzer. Using a two-fold cutoff value and a P-value less than 0.05, 696 genes were identified as differentially transcribed genes in AA-treated mice when compared with the controls (233 up-regulated and 463 down-regulated).

Gene ontology analysis revealed that the principle pathways affected by AA were xenobiotic metabolism by cytochrome P450 (CYPs) and glutathione metabolism. The authors note a discrepancy between the amount of CYP 2E1 transcript measured by PCR and by microarray, with increased transcript observed in the latter but not the former. The authors suggest this to be the result of saturation of the microarray signal in both the control and treated animals.

Whilst this study does not indicate an increase in DNA damage response gene transcription, micronucleus formation and mutagenicity was observed at this dose level, as reported by Manjanatha *et al.* (2006). This suggests that changes in the level of mRNA transcripts, as measured in this experiment, are not able to detect the genetic damage occurring at this dose level.

Zhang X, Jiang L, Geng C, Yoshimura H, Zhong L. (2008) *Inhibition of acrylamide genotoxicity in human liver-derived HepG2 cells by the antioxidant hydroxytyrosol. Chem Biol Interact. [Epub ahead of print]*

An *in vitro* micronucleus assay was performed in Hep2G cells, pretreated with increasing concentrations of the antioxidant hydroxytyrosol and exposed to acrylamide (625 μ M, 24 h). Slides were prepared and 1000 binucleated cells per treatment concentration were scored. Acrylamide exposure resulted in a significant increase in micronucleated cells ($p < 0.01$) and the antioxidant resulted in a significant reduction in acrylamide induced micronucleated cells ($p < 0.01$). This study may indicate that, at these relatively high doses, in this model system (which may not be capable of metabolising acrylamide to glycidamide) a component of the observed genotoxicity may be related to reactive oxygen species.

Lamy E, Völkel Y, Roos PH, Kassie F, Mersch-Sundermann V. (2007) *Ethanol enhanced the genotoxicity of acrylamide in human, metabolically competent HepG2 cells by CYP2E1 induction and glutathione depletion. Int J Hyg Environ Health 211(1-2):74-81.*

An *in vitro* comet assay was performed in Hep2G cells treated with acrylamide (1.25-10 mM, 24 h), with a concentration related increase in olive tail moment which was significant at 2.5 mM and above. Pre-treatment with increasing concentrations of the CYP2E1 inducer ethanol (15-250 mM, 24h) alone did not cause increased DNA damage; however, pre-treatment with ethanol (15-250 mM, 24h) followed by exposure to acrylamide (5 mM, 24 h) resulted in an ethanol concentration related increase in DNA damage. Limited Western blot data demonstrated that CYP2E1 protein could not be detected in untreated cells and ethanol treatment resulted in a modest increase in CYP2E1 protein level. This study suggests that a component of the genotoxicity of acrylamide may be related to induction of CYP 2E1 in this test system; although glutathione depletion as a result of the combined exposure to acrylamide and ethanol might also contribute to the observed increase in DNA damage.

Dobrzyńska MM. (2007) *Assessment of DNA damage in multiple organs from mice exposed to X-rays or acrylamide or a combination of both using the comet assay. In Vivo. 21(4):657-62.*

Comet assays were performed on preparations from the bone marrow, spleen liver, kidney, lungs and testes of mice exposed to acrylamide (50, 75, 100 or 124 mg/kg bw). Statistically significant dose-related increases in DNA damage were observed in all tissues.

El-Rahim WM, Khalil WK, Eshak MG. (2008) Genotoxicity studies on the removal of a direct textile dye by a fungal strain, in vivo, using micronucleus and RAPD-PCR techniques on male rats. J Appl Toxicol 28(4):484-90.

Groups of 5 Male rats were administered acrylamide (30 mg/kg bw/day, oral gavage, 2 or 8 weeks) as a positive control. 3000 polychromatic erythrocytes (PCE) per animal were evaluated for micronucleus formation. There was a significant increase over untreated control at both time-points. RAPD (Random Amplified Polymorphic DNA) fingerprints were prepared from genomic DNA (source tissue not stated). An increase in polymorphic bands was noted although the authors do not offer interpretation of this result.

Question asked of the Committee

What conclusions can be drawn from the 5 recently published papers (addendum to MUT/08/15) and how should these conclusions be reflected in the draft Statement?

**COM Secretariat
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