

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

Review of the Genotoxicity of Acrylamide

Secretariat Acrylamide Search post July 2007

[This discussion document has been drafted to aid members in their consideration of acrylamide. It does not represent a formal view of COM]

In order to bring the secretariat search up to date, the Pubmed database was interrogated using the following search term:

(acrylamide OR glycidamide) AND ("DNA damage" OR "DNA adduct" OR aneugen OR clastogen* OR genotoxic* OR mutagen*)*

18 additional references identified.

2 of these references were identified by COM members (Mei *et al.* 2007, Jiang *et al.* 2007). Mei was included in the systematic review. Jiang *et al.* provide evidence for a possible oxidative DNA damage mechanism.

2 references have been identified by the PPG (Witt *et al.* 2008, and Clement *et al.* 2007). Witt *et al.* present a comparison of micronucleus assays using flow cytometry and microscopy based scoring, showing that the results are comparable. Clement *et al.* (2007) present an expression profile of glycidamide treated cells, although the relevance of these results is hard to determine.

2 further references have been identified. Lamy *et al.* (2007) investigated the effects of ethanol (an inducer of CYP2E1) on acrylamide related DNA damage by comet assay, reporting evidence of synergy. Wolf *et al.* (2007) present part of the validation of the hen egg micronucleus test, with a positive result for acrylamide.

The abstracts of these 6 references are provided for Members' consideration.

The remaining 12 references were excluded for the following reasons: 4 reviews, 2 papers discussing acrylamide chemistry, 1 epidemiology paper, 2 papers using fluorescence quenching methodology, 1 modelling acrylamide data, and 2 using (2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide) as a positive control.

Acrylamide Mutagenicity Review - Abstracts (Post July 2007)

CLEMENT2007: Clement FC, Dip R, Naegeli H. (2007) Expression profile of human cells in culture exposed to glycidamide, a reactive metabolite of the heat-induced food carcinogen acrylamide., *Toxicology* 240(1-2):111-124

Recent findings of acrylamide in many common foods have sparked renewed interest in assessing human health hazards and the long-term risk associated with exposure to vinyl compounds. Acrylamide is tumorigenic at high doses in rodents and has been classified as a probable human carcinogen. However, cancer risk projections in the population remain problematic because the molecular pathogenesis of acrylamide at the low level of dietary uptake is not understood. In particular, the question of whether specific transcriptional responses may amplify or mitigate the known genotoxicity of acrylamide has never been examined. Here, we used high-density DNA microarrays and PCR validations to assess genome-wide messenger profiles induced by glycidamide, the more reactive metabolite of acrylamide. The expression changes resulting from glycidamide treatment of human epithelial cells are characterized by the induction of detoxification enzymes, several members of the glutathione system and antioxidant factors. Low-dose experiments indicate that the up-regulation of epoxide hydrolase 1 represents the most sensitive transcriptional biomarker of glycidamide exposure. At higher concentrations, glycidamide induces typical markers of tumor progression such as steroid hormone activators, positive regulators of nuclear factor-kappaB, growth stimulators and apoptosis inhibitors. Concomitantly, growth suppressors and cell adhesion molecules are down-regulated. The main implication of these findings for risk assessment is that low concentrations of glycidamide elicit cytoprotective reactions whereas transcriptional signatures associated with tumor progression may be expected only at doses that exceed the range of ordinary dietary exposures.

JIANG2007: Jiang L;Cao J;An Y;Geng C;Qu S;Jiang L;Zhong L; (2007) Genotoxicity of acrylamide in human hepatoma G2 (HepG2) cells, *Toxicol In Vitro* 21(8):1486-1492

The recent finding that acrylamide (AA), a carcinogen in animal experiments and a probable human carcinogen, is formed in foods during cooking raises human health concerns. The relevance of dietary exposure for humans is still under debate. The purpose of the study was to evaluate the possible genotoxicity of acrylamide in human hepatoma G2 (HepG2) cells, a cell line of great relevance to detect genotoxic/antigenotoxic substances, using single cell gel electrophoresis (SCGE) assay and micronucleus test (MNT). In order to clarify the underlying mechanism(s) we evaluated the intracellular generation of reactive oxygen species (ROS) and the level of oxidative DNA damage by immunocytochemical analysis of 8-hydroxydeoxyguanosine (8-OHdG). The involvement of glutathione (GSH) in the AA-induced oxidative stress was examined through treatment with buthionine sulfoximine (BSO) to deplete GSH. The results indicate that AA caused DNA strand breaks and increase in frequency of MN in HepG2 cells in a dose-dependent manner. The possible mechanism underlies the increased levels of ROS, depletion of GSH and increase of 8-OHdG formation in HepG2 cells treated with AA. We conclude that AA exerts genotoxic effects in HepG2 cells, probably through oxidative DNA damage induced by intracellular ROS and depletion of GSH

LAMY2007: Lamy E;Volkel 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*

In the present study, the genotoxicity of acrylamide (AA) was investigated in HepG2 cells using SCGE. Additionally, the influence of ethanol on the modulation of AA-induced DNA-migration caused by CYP2E1-upregulation and/or GSH-depletion was examined in the same cell line. For the ethanol/AA combination assays, the cells were treated with ethanol for 24h prior to exposure to 5mM AA for another 24h. 1.25 to 10mM AA-induced DNA migration (OTM) in HepG2 cells in a concentration-dependent manner, e.g., exposure to 10mM AA, resulted in an 8-fold increase of DNA migration compared to the negative control. Treatment with 120mM ethanol prior to exposure to 5mM AA increased the level of DNA migration more than 2-fold as compared to cells treated with 5mM AA alone. Immunoblotting showed a clear ethanol-induced increase of CYP2E1, which plays a pivotal role in AA toxication. Additionally, intracellular GSH levels were significantly reduced after ethanol or AA treatment. In the ethanol/AA combination experiments, GSH depletion was comparable to the additive effect of the single compounds. No induction of apoptosis (ssDNA assay), but necrosis was identified as responsible for the reduction of viability with increasing compound concentration. The data clearly show a higher genotoxic potential of ethanol/AA combination treatment compared to AA treatment alone. In conclusion, both the ethanol-mediated induction of CYP2E1 and the depletion of GSH provide a mechanistic explanation for the over-additive effects of ethanol and AA. Even though the concentrations used in this study were rather high, consequences for the dietary intake of AA-containing food and alcoholic beverages should be discussed

MEI2008: Mei N;Hu J;Churchwell MI;Guo L;Moore MM;Doerge DR;Chen T; (2008) Genotoxic effects of acrylamide and glycidamide in mouse lymphoma cells, *Food Chem Toxicol* 46(2):628-636

In addition to occupational exposures to acrylamide (AA), concerns about AA health risks for the general population have been recently raised due to the finding of AA in food. In this study, we evaluated the genotoxicity of AA and its metabolite glycidamide (GA) in L5178Y/Tk(+/-) mouse lymphoma cells. The cells were treated with 2-18mM of AA or 0.125-4mM of GA for 4h without metabolic activation. The DNA adducts, mutant frequencies and the types of mutations for the treated cells were examined. Within the dose range tested, GA induced DNA adducts of adenine and guanine [N3-(2-carbamoyl-2-hydroxyethyl)-adenine and N7-(2-carbamoyl-2-hydroxyethyl)-guanine] in a linear dose-dependent manner. The levels of guanine adducts were consistently about 60-fold higher across the dose range than those of adenine. In contrast, no GA-derived DNA adducts were found in the cells treated with any concentrations of AA, consistent with a lack of metabolic conversion of AA to GA. However, the mutant frequency was significantly increased by AA at concentrations of 12mM and higher. GA was mutagenic starting with the 2mM dose, suggesting that GA is much more mutagenic than AA. The mutant frequencies were increased with increasing concentrations of AA and GA, mainly due to an increase of proportion of small colony mutants. To elucidate the underlying mutagenic mechanism, we examined the loss of heterozygosity (LOH) at four microsatellite loci spanning the entire chromosome 11 for mutants induced by AA or GA. Compared to GA induced mutations, AA induced more mutants whose LOH extended to D11Mit22 and D11Mit74, an alteration of DNA larger than half of the chromosome. Statistical analysis of the mutational spectra revealed a significant difference between the types of mutations induced by AA and GA treatments ($P=0.018$). These results suggest that although both AA and GA generate mutations through a clastogenic mode of action in mouse lymphoma cells, GA induces mutations via a DNA adduct mechanism whereas AA induces mutations by a mechanism not involving the formation of GA adducts

WITT2008: Witt KL;Livanos E;Kissling GE;Torous DK;Caspary W;Tice RR;Recio L; (2008) Comparison of flow cytometry- and microscopy-based methods for measuring micronucleated reticulocyte frequencies in rodents treated with nongenotoxic and genotoxic chemic

The development of automated flow cytometric (FCM) methods for evaluating micronucleus (MN) frequencies in erythrocytes has great potential for improving the sensitivity, reproducibility, and throughput of the traditional *in vivo* rodent MN assay that uses microscopy-based methods for data collection. Although some validation studies of the FCM evaluation methods have been performed, a comprehensive comparison of these two data collection methods under routine testing conditions with a variety of compounds in multiple species has not been conducted. Therefore, to determine if FCM evaluation of MN frequencies in rodents was an acceptable alternative to traditional manual scoring methods in our laboratory, we conducted a comparative evaluation of MN-reticulocyte (MN-RET) frequencies determined by FCM- and microscopy-based scoring of peripheral blood and bone marrow samples from B6C3F1 mice and Fisher 344 rats. Four known inducers of MN (cyclophosphamide, ethyl methanesulfonate, vincristine sulfate, acrylamide) were assayed in bone marrow and peripheral blood of both mice and rats. In addition, MN-RET frequencies were measured in bone marrow (microscopy) and peripheral blood (FCM) of mice treated with five nongenotoxic chemicals (S-adenosylmethionine chloride, cefuroxime, diphenolic acid, 3-amino-6-methylphenol, pentabromodiphenyl oxide). No significant differences were observed between results obtained by the two methods in either species. These results support the use of FCM for determining MN-RET frequency in rodents after chemical exposure

WOLF2007: Wolf T;Niehaus-Rolf C;Banduhn N;Eschrich D;Scheel J;Luepke NP; (2007) The hen's egg test for micronucleus induction (HET-MN): Novel analyses with a series of well-characterized substances support the further evaluation of the test system, Mutat

The hen's egg test for micronucleus induction (HET-MN) combines the use of the commonly accepted genetic endpoint 'formation of micronuclei' with the well-characterized and complex model of the incubated hen's egg, which enables metabolic activation, elimination and excretion of xenobiotics-including those that are mutagens or promutagens. This assay procedure is in line with demands for animal protection. In three previous publications we presented the scientific rationale and methodological aspects for this assay as well as results for some well-characterized mutagens and promutagens. Here we present the results of new experiments involving further genotoxic and non-genotoxic model substances. Making a comparison with published data we have to date not found any false negatives or false positives in the experiments presented here and in trials published before, thus demonstrating a promising predictivity of genotoxic effects with this assay. We could confirm relevant genotoxicity for the following substances in the HET-MN: acetylaminofluorene (2-AAF), acrylamide (ACM), cytarabine (AraC), methotrexate (MTX), cadmium chloride (CD), dipotassium monochromate (DPC), and epirubicine (EPI). Negative results were obtained for azorubin (E122), orange G (OG) and starch (STRC). The micronucleus frequencies (MNE II) of the concurrent negative controls were in agreement with the values of the historical negative control (0.87 per thousand \pm 0.87; average \pm s.d.). This value is based upon the scoring of 556,500 erythrocytes from 445 eggs. In historical positive controls the administration of 0.05mg cyclophosphamide/egg at d8 resulted in an MNE II-frequency of 12.4 per thousand \pm 6.8 (average \pm s.d.) at d 10.5. This value is based upon the scoring of 249,250 erythrocytes from 223 eggs