

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

DRAFT

MUT/08/2

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

REVIEW OF GENOTOXICITY OF ACRYLAMIDE

OVERVIEW OF GENOTOXICITY OF ACRYLAMIDE SINCE 1995

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

Referral to COM on acrylamide

1. The HSE requested a further evaluation from the COM regarding the information cited by the PPG in its letter to the chair of COM (dated 8 May 2007) (Annex 2 to this draft discussion paper). The Food Standards Agency have also requested that a consideration be given to all available genotoxicity data on acrylamide by COM. The COM agreed that the EU risk assessment review completed by HSE (EU Risk Assessment report 2002) could be used as a basis for the review.

Background to COM review of acrylamide

2. HSE asked for an opinion on the evidence regarding germ cell mutagenicity of acrylamide and the evidence regarding a threshold for germ cell mutagenicity with this chemical in January 2007. A response to HSE was published in February 2007^a. The COM was made aware of a response from the Polyelectrolyte Producers Group (PPG) to the chair (dated 8 May 2007) at the COM meeting of the 17 May 2007 and agreed to a further evaluation of the genotoxicity data on acrylamide.

3. The COM considered a presentation from PPG on 'an analysis of the genotoxicity of acrylamide' at the October 2007 COM meeting. Additional data submitted by PPG following that meeting is presented in MUT/08/01.

4. The COM secretariat drafted an overview of the EU Risk Assessment of acrylamide and outlined a strategy for the COM review of published literature in MUT/07/17. The COM agreed that the EU risk assessment could form the basis of literature reviewed up to 1995, and that COM secretariat overview would focus on published literature from 1995 onwards. The Com agreed also agreed to review a number of specific research papers published prior to 1995 which had been identified by the secretariat but not included in the EU risk Assessment report. A reference list for the current review was subsequently distributed to COM members, A small number of newly published additional references were identified by COM members.

^a <http://www.advisorybodies.doh.gov.uk/com/acryla.htm>

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Introduction to COM review

5. Summaries of the reviewed papers for acrylamide and glycidamide can be found in Table 1 Annex 1:

Study Type	Page
In Vitro Gene Mutation (Bacterial)	1
In Vitro Gene Mutation (Mammalian)	2
In Vitro Cell Transformation	8
In Vitro Chromosomal Aberrations	9
In Vitro Micronucleus	11
In Vitro DNA Damage	13
In Vitro DNA Adducts	21
In Vitro DNA Adducts (Cultured Cells)	23
In Vivo DNA Adducts	25
In Vivo Gene Mutation	33
In Vivo Chromosomal Aberrations	36
In Vivo Micronucleus	38
In Vivo DNA Damage	46
In Vivo DNA Synthesis	48
In Vivo Human Biomonitoring	50
In Vivo Germ Cell Mutation	51
In Vivo Germ Cell Dominant Lethal	54
In Vivo Germ Cell Embryo Abnormalities	59
In Vivo Germ Cell Chromosomal Aberrations	60
In Vivo Embryo Micronucleus	63
In Vivo Germ Cell DNA Adducts	64
In Vivo Germ Cell UDS	65
In Vivo Germ Cell Other	66
In Vivo Gene Mutation (Drosophila)	67

6. The structure of the summaries have been ordered so that data from tests can be viewed together (e.g. bacterial or mammalian cell) and information on end point(s) studied (e.g. gene mutation, chromosomal aberrations and aneugenicity) is clear. The COM also asked for specific information from each paper on role of metabolism and mechanism for end points studied should be identified.

7. Table 2 in Annex 1 presents a overview of all the data on acrylamide and glycidamide reviewed indicating study type, end point and results (positive or negative).

8. The following narrative review is provided to help the Committee evaluate whether conclusions for each test/endpoint would differ from the EU risk assessment report. As Members requested, an attempt to evaluate the

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

role of metabolism and mechanism(s) of acrylamide was made. The purpose of evaluating data on glycidamide was to investigate the genotoxicity which might be due to metabolism of acrylamide to glycidamide in metabolically competent cells.

9. Selected relevant excerpts from each study is presented in Annex 2, where studies are arranged in alphabetical order. A full copy of all papers included in the systematic review will be available at the meeting and can be provided to Members upon request.

10. No specific review of acrylamide toxicokinetics was identified post 1995 in the searches. The secretariat have therefore appended the papers by Doerge on toxicokinetics in mice and rats in Annex 3.

Overview of genotoxicity of acrylamide

11. The secretariat has reviewed the individual studies to identify test material purity. Thus acrylamide is generally sourced from commercial manufacturers and is often of high grade purity ($\geq 99\%$). Glycidamide in a number of papers is synthesised. Generally whether synthesised or obtained from commercial sources glycidamide is $\geq 95\%$ pure with approximately 1% acrylamide present.

In vitro Mutagenicity, DNA Damage (Table 1, Annex 1)

In vitro Gene Mutation (Bacterial)

12. Data reviewed in the EU risk assessment report concluded that negative results have been reported in standard bacterial mutagenicity tests both in presence and absence of exogenous metabolic activation. Yang et al. (2005) reported a positive response in *Salmonella typhimurium* TA 98 in presence or absence of exogenous metabolic activation and a positive result in TA 100 in the presence of exogenous metabolic activation. These data would appear to contrast with the data reviewed in the EU risk assessment report.

13. Emmert B *et al.* (2006) did not record a positive response in *Salmonella typhimurium* YG7108pin3ERb5 (which can metabolise compounds via CYP2E1). It is noted that a number of compounds which should have been activated with mutagenic effects reported also gave negative results suggesting the test system was not adequate.

Question 1: Does the COM consider bacterial mutagenicity studies alter the conclusions reached in the EU risk assessment report? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

In vitro Gene mutation (Mammalian)

14. Data reviewed in the EU risk assessment report concluded that acrylamide is a direct acting mutagen in the available assays probably causing clastogenic effects. Mei N *et al.* (2007) showed an increase in Tk mutant frequency L5178Y TK+/- cells in the absence of exogenous metabolic activation, suggesting at this was the result of a clastogenic mode of action. In mammalian cell tests for gene mutation equivocal results were obtained in V79 cells for *hprt* (Baum M *et al.* 2005) and a negative result was obtained in TK6 cells (Koyama 2006). The most relevant positive finding for gene mutation has been reported by Besaratinia A and colleagues in two studies (Besaratinia A *et al.* 2003, 2004) which reported increased *cII* mutation in Big Blue mouse fibroblasts. It is noted that the cells may have had some metabolic competency. The authors conclude that metabolism of acrylamide to glycidamide was most likely responsible for the effects documented.

Question 2: Does the COM consider these mammalian mutagenicity studies alter the conclusions reached in the EU risk assessment report? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

In vitro cell transformation

15. A number of positive results have been reported in cell transformation assays both in presence and absence of exogenous metabolic activation were reported in the EU risk assessment report. Park J *et al.* (2002) reported a positive finding in SHE cells. It is noted that the effects were observed when a non specific P-450 inhibitor was used. The authors suggest acrylamide induced a clastogenic effect responsible for the cell transformation and that GSH depletion was partly responsible for this effect.

Question 3: Cell transformation tests are generally not used by COM in deriving conclusions with regard to *in vitro* genotoxic activity. Do members have any comments?

In vitro chromosomal aberrations (mammalian cell)

16. Data reviewed in the EU risk assessment report concluded that acrylamide is a direct acting clastogen in mammalian cells *in vitro*. Yang HJ *et al.* (2005) reported a dose related clastogenic effect of acrylamide in CHL cells in the presence and absence of exogenous metabolic activation, although a non-standard 22 h incubation with S9 was reported. Martins *et al.* reported a positive result for chromosomal aberrations in V79 Mz cells (which were reported to lack CYP2E1 activity). It was suggested that acrylamide mediated its effects via a Michael-type reaction or via free radical formation (see comments on DNA adduct formation below). The maximal effect in this assay was reported at 2 mM. Glatt reported a positive result for SCE formation in V79 cells containing transfected CYP2E1 which was greater than the effect reported in V79 Mz cells. Thus overall there is evidence for both

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

direct effects of acrylamide and effects mediated by glycidamide. The mechanism for the direct effects of acrylamide is unknown.

Question 4: Does the COM consider these clastogenicity studies alter the conclusions reached in the EU risk assessment report? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

In vitro Micronucleus tests (mammalian cell)

17. No *in vitro* micronucleus assays were reported in the EU risk assessment report. Baum M *et al.* (2005) reported negative findings for Mn induction using the CBMN assay in whole blood cultures from healthy donors. Koyama N reported an equivocal response for MN induction in TK6 cells. A positive result for MN induction was reported by Jie *et al.* (2001) who used FISH to analyse MN by acrylamide in NIH3T3 cells. There was evidence for a variety of mechanisms including clastogenicity, aneugenicity and effects on telomeres. There was no information on the endogenous metabolic activity in NIH3T3 cells.

Question 5: Do members have any comments on these micronucleus studies? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

In vitro DNA damage (mammalian cells)

18. The EU risk assessment review concluded that the available studies (UDS assays) were inconsistent and it was therefore difficult to draw a definite conclusion. Ma XX *et al.* (1996) investigated comet response in kertainocyte cell line HaCaT cells. A positive response was documented which was reduced by incubation with the pan P450 inhibitor 1-aminobenzotriazole. The authors conclude that the response in this test system was due to oxidative metabolites formed from acrylamide (abstract available only). Blasiak J *et al.* (2004) reported a dose related increase in DNA damage (alkaline comet assay) in PBLs from a healthy male donor. Post treatment of cell with repair enzymes recognising alkylated bases increased the response indicating DNA alkylation. Pre-treatment with compounds/vitamins inhibiting free radical/oxygen species formation caused a decrease in DNA damage. Overall the mechanism of effects was unclear. It was noted that extent of acrylamide metabolism by PBLs was unclear. A negative result for DNA damage was reported by Baum using whole blood cultures from three male healthy donors. (Baum M *et al.* 2005). Puppel *et al.* (2005) reported a positive response for DNA damage in V79 cells and Caco-2 cells at relatively high concentrations (6 mM acrylamide) but not in rat hepatocytes. It was noted that treatment with BSO (to reduce glutathione levels) increased the DNA damage. V-79 cells and Caco-2 cells were not expected to have any CYP2E1 activity and thus the effects in this study were mediated by acrylamide. The mechanism for the DNA damage in this study was unclear. Evidence for DNA damage was

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

documented in primary thyroid cells from dog, sheep and humans and in rat thyroid cell line PC13 and FRTL5 cells using alkaline comet assay. The impact of metabolism in these cells and mechanisms of effects was not studied (Chico G *et al.* 2006). Negative findings for the comet assay were reported in TK6 cells (at concentrations up to 14 mM). (Koyama N *et al.* 2006).

19. Thus acrylamide induced DNA damage in mammalian cells in comet assays. The effects were mediated both by acrylamide and metabolism to glycidamide. There was evidence for a range of effects including alkylation and free radicals.

Question 6: Does the COM consider these DNA damage studies alter the conclusions reached in the EU risk assessment report? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

Summary *In vitro* data (acrylamide).

20. The available data are consistent with a lack of mutagenic effect of acrylamide in bacteria. The one positive result identified post 1995 needs further clarification and confirmation before the data can be incorporated into the evaluation of acrylamide. The available data support the conclusion that acrylamide is mutagenic in mammalian cells inducing gene mutations, clastogenicity and aneugenicity, and causes DNA damage in mammalian cells. These effects occur through a number of mechanisms which have not been fully elucidated via direct effects of acrylamide or via metabolism to glycidamide.

DNA adduct formation *in vitro*, in cultured cells and *in vivo*

In vitro reactivity with DNA and free nucleotides

21. The formation of acrylamide related adducts has been investigated in *in vitro* experiments. As reported in the EU risk assessment report, Solomon *et al.* 1985 (Cancer Res. 45:3465-70) found direct adduction of acrylamide to calf thymus DNA when incubated at pH 7 for 40 days at 37°C. 2-carboxyethyl adducts were detected at N1 and N6 of 2'-deoxyadenosine, N1 of 2'-deoxyguanosine and N3 of 2'-deoxycytidine. 2-formamidoethyl adducts at N7 of 2'-deoxyguanosine were also detected. These experiments only produced adducts after long incubation (40 days) with high acrylamide concentration (68 mM).

22. Subsequent to the EU risk assessment literature search, Sergerbäck *et al.* (1995) reported detection of the N7-(2-carbamoyl-2-hydroxyethyl)guanine (N7-GA-Gua) adduct following incubation of radiolabelled acrylamide in the presence of un-induced rat S9 liver homogenate for 2 hours at 37°C. Several subsequent *in vitro* experiments with isolated DNA or free nucleotides treated

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

with glycidamide have identified a range of adducts: N7-GA-Gua, N1-GA-Gua, N3-GA-Ade, N1-GA-dA, N6-GA-dA, N3-GA-dT and N3-GA-Cyd.

23. Gamboa da Costa *et al.* (2003) developed methods for detecting the relevant adducts. As reported in later publications from these researchers, the limit of detection (LOD) for N7-GA-Gua and N3-GA-dA was in the region of 1 adduct in 10^8 nucleotides, with a limit of quantification (LOQ) approximately 0.5 adducts in 10^8 nucleotides. It was not possible to detect N1-GA-dA adducts, even when converted to N6-GA-dA due to limits in method sensitivity.

DNA adducts in cultured cells

24. N7-GA-Gua and N3-GA-Ade adducts were found in cultured hamster fibroblasts (V79) treated with glycidamide. N3-GA-Ade adducts were present at levels 100-fold lower than N1-GA-Gua. N7-GA-Gua adducts were detectable in cells treated with acrylamide but with reduced potency (2mM was equivalent to 1 μ M glycidamide) and N3-GA-Ade were below the limit of detection (Martins *et al.* 2006). A recent publication from the same group using L51y8Y/Tk+/- cells found a similar 60-fold difference between N7-GA-Gua and N3-GA-Ade adducts, but could not detect either adduct in acrylamide treated cells (Mei *et al.* 2007).

25. Besaratinia *et al.* reported the formation of DNA adducts in Big Blue mouse embryonic fibroblasts and normal human epithelial cells which had been exposed to acrylamide and glycidamide. The cell lines may have some CYP2E1 activity. Polymerase blocking lesions were mapped by PCR but the chemical identity of the adducts was not determined. The formation of DNA adducts was dose-dependent, but there was no direct relationship between pattern of *c//* mutations and mapping of DNA adducts. In other studies by this group of researchers, DNA adducts formed following acrylamide treatment were reported to occur at similar locations in TP53 and *c//* to those formed from glycidamide.

DNA adducts *in vivo*

26. The EU risk assessment report cited evidence of DNA alkylation in the liver and, to a lesser extent, the testes. These studies were based on measuring DNA associated radioactivity. Sergerbäck *et al.* (1995) found N7-GA-Gua adducts in the liver and other organs of rats and mice following an intra-peritoneal dose of radiolabelled acrylamide. Levels were lower than those quoted in the EU risk assessment report. The authors comment that their method only quantified N7-GA-Gua and that much of the DNA associated radioactivity was not associated with specific adducts (it appeared in the void volume upon purification).

27. Gamboa da Costa *et al.* (2003) developed methods for quantifying N7-GA-Gua and N3-GA-dA adducts. Intra-peritoneal administration of acrylamide to mice resulted in quantifiable levels of N7-GA-Gua and N3-GA-dA adducts

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

(N7-GA-Gua being at a >70-fold higher level than N3-GA-dA). Administration of glycidamide resulted in slightly increased levels of adducts which was not consistently significant between studies and sex. Control mice were found to have low but detectable levels of N7-GA-Gua, which was probably the result of autoclave sterilisation of the rodent diet (Twaddle *et al.* 2004).

28. Subsequent studies compared intra-peritoneal dosing with oral gavage and dietary administration in mice and rats. These studies used low doses (0.1 mg/kg bw) which are closer to human mean dietary exposure, and ~500-fold lower than previous adduct studies (Doerge *et al.* 2005a, b & c).

Notably, these studies found that dietary administration slightly attenuated acrylamide bioavailability but that oral administration resulted in more extensive metabolism to glycidamide, probably the result of first pass metabolism. Furthermore, the levels of N7-GA-Gua adducts were proportional to the internal exposure to glycidamide (AUC) regardless of sex, species or chemical. This equated to ~2 adducts per AUC unit ($\mu\text{M} \times \text{h}$).

29. N7-GA-Gua and N3-GA-dA adduct levels were analysed in a range of tissues in rats and mice. Consistent with previous data, the N3-GA-dA adduct was present at ~70-fold lower levels than N7-GA-Gua. The authors comment that differences in adduct levels alone are not sufficient to account for the tissue specificity of the tumours in the carcinogenicity studies. Although the authors note that limits of method sensitivity meant they could not examine the N1-GA-dA adduct, which has the potential to cause mis-coding.

30. Doerge *et al.* (2005 a, b & c) also reported on the kinetics following gavage dosing of rats and mice. They found that acrylamide elimination had a similar rate constant to glycidamide formation, and glycidamide elimination had a similar rate constant to N7-GA-Gua adduct formation. Sub-chronic administration of acrylamide (~1 mg/lg bw/day for 28 days) to mice in drinking water showed an accumulation of N7-GA-Gua adducts in liver reaching a steady state of (3-400 adducts per 10^8 nucleotides) at 14 days. Similar data was obtained in rats although the levels slowly declined from the 14 day maximum in male rats.

31. Ghanayem *et al.* (2005) compared adduct formation in CYP2E1 knockout mice following intra-peritoneal administration of acrylamide (50 mg/kg). N7-GA-Gua adducts were present in a 100-fold excess to N3-GA-dA adducts in treated wild-type mice. However, treated CYP2E1 mice had detectable levels of the N7-GA-Gua adduct, albeit at lower (>50-fold) levels than wild-type mice. The authors suggest this shows that ~2% of acrylamide may be converted to glycidamide by a non CYP2E1 mediated.

32. Tareke *et al.* (2006) compared N7-GA-Gua adduct levels, haemoglobin adduct levels and internal glycidamide exposure, in rats and mice following sub-chronic administration in drinking water. They found the three parameters were each significantly associated with the others, suggesting that haemoglobin adducts may be a useful biomarker of liver adduct levels.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Attempts to model the data from rodent to human indicate liver adduct rates may be in the region of 0.06 to 0.3 adducts per 10^8 nucleotides.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

In vivo germ cell DNA adducts

33. Holland N *et al.* (1999) reported the formation of DNA adducts in sperm from mouse caudal epididymides using AMS analysis following dosing of 50 mg/kg bw. Xie *et al.* (2006) administered between 0.075-1000 µg/kg bw i.p. Sperm DNA was isolated and AMS analysis used to investigate DNA adducts. A plot of log acrylamide adducts (ng/g) against log acrylamide dose showed a linear relationship for haemoglobin, serum albumin, protamine, sperm head and tail adducts, and sperm DNA adducts. It was noted that a dose level of two orders of magnitude was needed to achieve DNA adducts equivalent to protein adducts. For DNA adducts, a linear dose-response was noted above 7.5 µg/kg bw. Presumably adduct levels below this dose were below the limit of detection for this assay.

Summary of DNA adduct data

34. Whilst acrylamide has been shown to only weakly react with DNA *in vitro*; its reactive epoxide metabolite glycidamide can form a range of adducts. The studies cited in the EU risk assessment report used DNA associated radioactivity as a measure of adducts following treatment with radiolabelled acrylamide. The subsequent data generally specifically quantifies levels of the N7-GA-Gua and N3-GA-Ade adducts, the former consistently occurring at levels ~60 to 100-fold greater than the latter. These adducts can be detected even at exposures close to the human mean dietary exposure; with evidence that acrylamide is more effectively metabolised to glycidamide at these low doses, that interspecies and sex differences are reduced. Problems with method sensitivity mean that it is not possible to quantify the N1-GA-dA adduct, which has the potential to cause mis-coding. It is interesting to note tissue differences in detected DNA adduct levels alone cannot explain the tissue specificity of the tumours found in the carcinogenicity studies.

35. N7-GA-Gua adducts appear to be related to the internal exposure (AUC) to glycidamide, either as a result of direct dosing with glycidamide, or forming as a result of acrylamide metabolism. Kinetic experiments suggest acrylamide elimination is related to glycidamide formation, and glycidamide elimination is related to N7-GA-Gua adduct formation. Interestingly, the N7-GA-Gua adduct can be detected in CYP2E1 knockout mice suggesting that there may be other pathways involved in glycidamide formation in these animals.

Question 7: Do COM members have any comments on these DNA adduct studies? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

In vivo mutagenicity and DNA damage in somatic cells.

In vivo Gene Mutation

36. A positive result was noted in a LacZ transgenic mutation assay ($62-89 \times 10^6$ cf $15-26 \times 10^6$ in controls) where groups of mice were given 5 daily i.p. dose of 50 mg/kg bw acrylamide and bone marrow samples examined for mutation frequency on days 3, 7 and 10 post dose. (Hoorn *et al.* 1993 and Mhyr 1991) The EU risk assessment report considered the full significance of the un-validated assay reviewed was unclear. Negative results were reported in Muta™ Mouse liver using lac-galE for mutant selection and doses of 50 or 100 mg/kg bw i.p. (in distilled water). Groups of 3-5 animals were dosed and killed at 3, 10 or 100 days post dose. Ethylnitrosourea gave a positive result (2-7 fold increase in mutation frequency). A slight increase in mutation frequency at 50 mg/kg bw was interpretable given the negative findings at 100 mg/kg bw. (Krebs O and favour J 1997).

37. In a separate study by Manjanatha *et al.*, groups of Big Blue male and female mice were given acrylamide in the drinking water at 100 mg/l or 500 mg/l for 7d/week for 4 weeks. It was noted that animals in the high dose group developed hind leg paralysis and sluggish movement and this dose level was halted after 3 weeks. These dose levels equated to approximately 19-25 mg/kg bw acrylamide and 88-111 mg/kg bw acrylamide. Spleen samples were taken for *hprt* mutation assay. The mean increase in mutation frequency was three fold and 16 fold at the low and high dose respectively. For liver *cII* mutation assay genomic DNA was extracted. A slight but not statistically significant increase in mutation frequency was documented in females and a two fold increase (statistically significant $P < 0.05$) increase in mutation frequency was documented in both sexes at the high dose level. Analysis of mutation spectra for the *cII* gene for 57 mutant plaques from acrylamide treated compared to 52 independent mutations in controls reported a significantly different spectrum for acrylamide treated animals. The majority of mutations were consistent with N7dG GA adducts (A-G transitions), but A-C and A-T transversions were consistent with N1 and N3 dA adducts. Overall the data were consistent with a gene mutation response of acrylamide mediated by metabolism to glycidamide.

38. A number of positive results using *Drosophila* assays have supplemented the studies recorded in the EU risk assessment report (Vogel EW 1993, Batiste-Alentom M *et al.* 1994, and 1995. A negative study was reported by Pontecorvo G and Fantaccione 2006). Overall the results of these studies are generally not used by COM in weight of evidence assessment of mutagenicity.

Question 8: Does the COM consider these data alter the conclusions reached in the EU risk assessment report? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

In vivo chromosomal aberrations

39. It was concluded in the EU risk assessment report that acrylamide produces chromosome aberrations in mice in bone marrow with limited evidence regarding splenocytes.

40. Kligerman *et al.* 1991 reported a positive response for chromosomal aberrations in spleen on C57BL/6 mice dosed by intraperitoneal injection with 100 mg/kg bw acrylamide. An increase in the number of hypoploid 2nd metaphase cells in bone marrow of male mice was documented following intraperitoneal dosing at 80 mg/kg bw (slight but not statistically significant) and at 120 mg/kg bw (approximate doubling which was statistically significant) (Gassner and Adler 1996). Nesterova EV *et al.* 1999 reported positive results for chromosome aberrations in bone marrow from BALB/c and C57BL/6 mice (sex not given) dosed i.p. at 100 mg/kg bw or 50 mg/kg bw/day for 5 days.

41. Krishna reported no evidence for chromosomal aberrations in bone marrow or spleen of rats dosed with 100 mg/kg bw acrylamide (route not given, only limited details available).

Question 9: Does the COM consider these *in vivo* clastogenicity studies alter the conclusions reached in the EU risk assessment report? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

In vivo micronucleus assays

42. The EU risk assessment report concluded that acrylamide produced MN in bone marrow and spleen in mice. There were 12 additional publications reporting data from micronucleus assays retrieved. The results have been briefly summarised below.

Publication	Dose/sampling	Result	Comment
Kligerman 1991	100 mg/kg bw, i.p 24h C57BL/6 male mice	Positive for splenocytes	
Schrivver-Schwemer 1997	125 mg/kg i.p 24h 102/E1xC3H/E1 male mice	Positive for aneugenicity and clastogenicity in PCEs	Aneugenicity assayed using FISH. Predominantly clastogenic effect.
Dobrynzka 2000	75 mg/kg bw i.p, 24,48,72h Pzh:Sfis mice (sex not given)	Negative for MN in bone marrow PCEs.	No positive control data.
Paulsson B <i>et al.</i> 2002	25,50,100 mg kg bw, i.p.48 h sample mice, 24h,48h sample rats	Linear dose-response for MN in peripheral blood erythrocytes in mice. Negative results in rats	Flow cytometric analysis. Hb adduct analysis suggested epoxide formation 3-6x in mice compared to rats.
Abramsson-Zetterberg 2003	2.5-100 mg/kg i.p. (21 dose levels). 1-30 mg/kg bw (6 dose levels) 42h sampling, CBA mice	Linear dose related increase in MN in peripheral blood erythrocytes.	Flow cytometric analysis.
Abramsson-Zetterberg 2005	Oral dosing to BALB/c mice blood sampling 42h.	No increase in MN in PCEs	Dose level not stated.
Ghanayem BI <i>et al.</i> 2005	25 or 50 mg/kg bw/day for 5 days. 24h	Significant dose-related increase in MN PCEs in wild type mice. No increase in MN in CYP2E1 null mice	Data suggests MN formation involved glycidamide formation.
Yang HJ <i>et al.</i> 2005	18-145 mg/kg bw by oral gavage, 48h sampling of peripheral blood. Male ICR mice	Significant increase in MN PCEs at 72.5 mg/kg bw and above	Assessed by conventional counting of stained slides.
Husoy T <i>et al.</i> 2005	50 mg/kg bw s.c, 42h cardiac puncture, in C57BL/6 or C57BL/6ApcMin/+ (Min-mice). C57BL/6ApcMin/+ (Min-mice) would be expected to have microtubule dysfunction.	An approximate 2 fold increase in MN PCEs was seen in both strains.	No conclusions can be reached regarding studies of DNA repair inhibition since no effects of acrylamide were noted in the concurrent investigations
Manjanatha MG <i>et al.</i> 2007	500 mg/l in drinking water (88-111 mg/kg bw/day for 7d/wk for 3 wks, Big Blue mice. 24 h sampling after	A significant increase in percentage MN reticulocytes was reported at the high dose level	Flow cytometry used.
Davis J and Reico L 2007	0.125 mg/kg bw/day-24. 0 mg/kg bw/day (10 dose levels) by oral gavage for 7days/week for 28 days. Blood sampled 24 hour after last dose. B6C3F1	Dose related increase in MN PCEs.	Flow cytometry used. Data fitted linear regression, quadratic regression and threshold models equally well when administered dose was used as the metric

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

43. The most relevant additional MN data post dating the EU risk assessment report is the increased evidence for a dose related effect in MN formation in PCEs in mice. Abramsson Zetterberg 2003 conclude a linear dose-related effect in mice based on administered intraperitoneal dose of acrylamide (21 dose levels used) and flow cytometric analysis. Paulsson *et al.* 2002 concludes a linear dose response for intraperitoneal administration in mice based on a much more limited number of dose levels. Yang *et al.* used oral dosing and reported evidence for a No Effect Level below 72.5 mg/kg bw using a single dose of acrylamide, 48h post dose sampling and conventional evaluation of MN PCEs on stained slides. Davis J and Reico L reported a dose related increase in MNPCEs in C57BL/6 mice given repeated oral doses of acrylamide for 28 days with a 24 h sample after the final dose (10 dose levels used). These authors report that the response was consistent with a number of dose-response relationships with an apparent threshold of effect.

Question 10: Does the COM consider these micronucleus studies alter the conclusions reached in the EU risk assessment report? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

In vivo human biomonitoring

44. A single human biomonitoring study published after 1995 provides no useful information on acrylamide genotoxicity (Kjuus H *et al.* 2005).

In vivo DNA damage assays

45. There were no DNA damage (comet) assays reported in the EU risk assessment report.

A dose-related positive alkaline comet response was reported in CYP2E1+/+ (wild type) mice in liver and in blood leukocytes following dosing i.p at 25 mg/kg bw/day or 50 mg/kg bw/day for 5 consecutive days. No comet response was recorded in CYP2E1 -/- mice (null). No effect was seen in the lung with either wild type or null mice. The authors considered that metabolism to glycidamide was responsible for the effects reported. It is noted the concurrent positive control urethane did not give a positive result in this test. (Ghanayem BI *et al.* 2005). A dose –related positive alkaline comet response was seen in bone marrow blast cells from BALB/c mice dosed with 5-50 mg/kg bw i.p acrylamide.

Question 11: Do COM members have any comments on DNA damage studies? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

In vivo DNA synthesis

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

46. In a study in male F344 and Sprague-Dawley rats, the effect of acrylamide in drinking water (2 or 15 mg/kg bw/day, for 28 days) on DNA synthesis in thyroid, adrenal medulla and testicular meosthelium were investigated. In a number of investigations the effect of 1-aminobenzotriazole (ABT) a P450 inhibitor was studied. Acrylamide induced DNA synthesis in all three tissues. The effect of ABT was less clear as in some studies it induced DNA synthesis by itself (eg in thyroid follicular cells). However overall the authors suggested that oxidative metabolism or glycidamide did not appear to exclusively account for the induction of DNA synthesis. The study was predominantly undertaken to investigate mechanisms of carcinogenesis in rat target organs. In general there was more evidence for an effect of the low dose used in F344 rats. (Lafferty JS *et al.* 2004) In a separate study Klaunig JE and Kamendulis LM (2005) measured DNA synthesis in F344 male rats dosed with 15 mg/kg bw/day via the drinking water for periods up to 28 days. An increase was seen in thyroid, testes, and adrenal medulla, but not in liver or adrenal cortex.

Question 12: Do members have any comments on these DNA synthesis studies with respect to the genotoxicity assessment of acrylamide? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

Conclusion; *In vivo* mutagenicity and DNA damage in somatic cells.

47. Acrylamide gave positive results in transgenic mutation assays in mice in liver, spleen and bone marrow. The evidence from Big Blue mice using *hprt* mutation in spleen and *cII* mutations in liver are consistent with a gene mutation response. Acrylamide induces chromosome aberrations and micronuclei formation in bone marrow and spleen of mice. There is evidence for a linear dose-response for MN formation in peripheral blood PCEs in mice following intraperitoneal dosing. There is evidence for a NOEL for MN formation in peripheral blood PCEs in mice given oral doses of acrylamide. There was no evidence for micronuclei formation in bone marrow of rats dosed with acrylamide. There are no useful human biomonitoring studies of genotoxicity of acrylamide available. The available comet assays in mice report positive results in liver and blood leukocytes. No comet response was seen in mice deficient in CYP2E1 suggesting that DNA damage in leukocytes is mediated via metabolism to glycidamide.

In vivo mutagenicity, DNA adduct formation and DNA damage in germ cells

In vivo Germ cell Mutation assays and Dominant lethal assays

48. In the EU risk assessment report, no evidence for a germ cell gene mutations was found in a preliminary validation *LacZ* assay at 50 mg/kg bw/d for 5 days (i.p).

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

49. A positive heritable translocation assay was reported in male C3H/EI mice given five daily dermal doses of 50 mg/kg bw and mated 1.5-8.5 days post exposure (Adler ID *et al.* 2004). Previous positive results for heritable translocation were documented in the EU risk assessment report from the same research group.

50. In the EU risk assessment report there are many positive dominant lethal assays in mice reported. Thus positive responses have been reported at dose levels of 3 mg/kg bw/day (oral) for 80 days, 25 mg/kg day (dermal) for 5 days and a single i.p dose of 125 mg/kg bw. The dominant lethal effects relate to effects on late spermatids and early spermatozoa. There are a number of studies either not reviewed in the EU risk assessment report or post dating the report which provide essentially consistent results with those reported in the EU risk assessment document. Thus positive dominant lethal assays were reported by Working PK *et al.* 1987 (in F344 rats), Dobrynska M (1990) in Pzh:SFISS mice, Tyl RW and Friedman (2003) in F344 rats and Adler 2004 in 102/E1xC3H/Ei)F1 mice.

51. A dominant lethal study was undertaken using CYP2E1+/+ (wild type) and CYP2E1-/- (null) male mice dosed with 12.5 or 25 mg/kg bw i.p for 5 days and then mated to untreated wild type females at various time points after dosing. The authors reported an effect of acrylamide on spermatids in wild type mice leading to a reduction in implants at both dose levels and confirmed the effect in a repeat experiment. There were no effects on percent pregnancy, mean number of implants per female, percent live fetuses/pregnant female or percent resorptions/pregnant female when CYP2E1 null males were treated with acrylamide. The authors concluded that glycidamide was the ultimate germ cell mutagen for dominant lethal effects binding to nucleophilic sites in chromatin in early spermatozoa. The precise mechanism was not elucidated.

52. Adler ID *et al.* investigated the dominant lethal effects of i.p dosing of 125 mg/kg bw in male (102/E1xC3H/E1)F1 mice in which some groups of animals were pretreated with ABT to inhibit metabolism of acrylamide to glycidamide. Four mating periods each of 4 days were used. ABT abolished the dominant lethal effect of acrylamide in the second mating. In the third mating there was a partial reduction in acrylamide induced dominant lethal effects. There was no effect of ABT in the fourth mating on the dominant lethal effects of acrylamide. The authors suggested that the dose of ABT may not have led to complete inhibition of acrylamide metabolism. The data are also consistent with a direct effect of acrylamide and an effect of glycidamide. The authors noted reduced fertility in the study may have been associated with an effect of acrylamide on the mobility of sperm mediated by an effect on motor proteins.

Question 13: Does the COM consider these mutation and dominant lethal studies alter the conclusions reached in the EU risk assessment report? What conclusions can be drawn regarding mechanism of action and the role of acrylamide metabolism?

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

In vivo Germ Cell Embryo Abnormalities

53. The morphology of embryos (blastocyst/morula) was examined from untreated female C57BL/6J mice mated to males given intraperitoneal injections of between 10-50 mg acrylamide /kg bw/day over 5 days. The animals were mated over a period of up to 5 weeks post dosing with acrylamide. Abnormal embryos (retarded cleavage, small number of blastomeres, embryos with lysis or abnormal cell structure and unfertilised eggs) predominated during weeks 1-3 mating at 40-50 mg/kg bw. In a separate experiment at 50 mg/kg bw/day, abnormal embryos were more frequent at week 2 compared to week 3 of mating. A dose –related effect was reported with an apparent NOEL of 10 mg/kg bw/day and 90% induction of abnormal embryos at 50 mg/kg bw/day. These data are largely consistent with the dominant lethal assays but suggest a lower NEL for i.p dosing. (Holland N *et al.* 1999).

In vivo germ cell chromosomal aberrations

54. Studies summarised in the EU risk assessment report documented evidence for chromosomal aberrations (including complex rearrangements) and aneuploidy in spermatogonia following either dietary administration (60 mg/kg bw/day for 1-3 weeks) or i.p dosing (100 mg/kg bw/day) to mice.

55. Male B6C3F1 mice were dosed i.p (5 x 50 mg/kg bw) and mated with untreated females at 2.5-48.5 days post final dose. Metaphase analysis of 1st cleavage division zygotes was undertaken. A post fertilisation reduction in the number of zygotes was reported on days 2.5-12.5 representing effects on late spermatids and early spermatozoa. Cell cycle delay was noted. Chromosomal aberrations were reported to be increased at up to day 27.5 (pachytene spermatocytes). A wide range of chromosomal aberrations was reported. The highest level of acentrics and translocations occurred on day 6.5. The highest level of unbalanced translocations occurred on day 9.5. The reported response for balanced translocations was reported to be similar to that documented in other studies. The authors suggested that the time interval for inheritable/dominant lethal mutagenicity extended from late spermatids and spermatozoa to pachytene spermatocytes (Marchetti F *et al.* 1997).

56. No evidence for an aneugenic effect was documented in a study where male (102/E1xC3H/E1)F1 mice were dosed with 60 mg/kg bw or 120 mg/kg bw acrylamide i.p and caudal epididymides obtained 22 days post dose and examined by FISH analysis for aneugenic effects on sex chromosomes and chromosome 8. A positive result was obtained with colchicine. (Schmid TE *et al.* 1999).

Question 14: Does the COM consider these germ cell chromosomal aberrations studies alter the conclusions reached in the EU risk assessment report? What conclusions can be drawn regarding mechanism of action?

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

In vivo Embryo Micronucleus

57. The EU risk assessment report documented evidence for micronucleus formation in spermatogonia and spermatocytes of rats. Male C57BL/6J mice were dose i.p with 50 mg/kg bw for 5 days and mated to untreated female C3H/J mice for 5-17 days after end of treatment. Females were sacrificed 86-88h after mating to sample post meiotic cells for examination of morphology and micronucleus formation. FISH staining was performed to analyse centromere DNA content of MN. The dose level used resulted in 10% loss of males at 24h post dose but no effect on mating. A large increase in the frequency of abnormal embryos (single cell, lysed blastomere and embryos with <10 cells (cf 65% of embryos had less than 10 cells). There was an increase in pyknotic and fragmented nuclei. There was a significant increase in micronuclei formation in treated normal and abnormal embryos. Both centromere positive and negative MN were increased in treated embryos.. The authors suggested both clastogenic and aneugenic mechanisms of action were involved in the observed response.(Titenko Holland N *et al.* 1998)

Questions 15: Does the COM consider these embryo micronucleus studies alter the conclusions reached in the EU risk assessment report? What conclusions can be drawn regarding mechanism of action?

Other in vivo germ cell assays

58. Male (102/E1xC3H/E1)F1 mice were dosed with 80 or 120 mg/kg bw acrylamide i.p. Microscopic examination using immunofluorescence stains for spindle structure was undertaken. A significant increase in spindle disturbances was identified which predominantly comprised multipolar spindles. The authors considered that the effects of acrylamide cannot be assigned to interactions with specific elements of the spindle but possibly could represent binding to various spindle proteins.

Question 16: Do members have any comments on these additional data? What conclusions can be drawn regarding mechanism of action?

Conclusion: *In vivo* mutagenicity and DNA damage in germ cells

59. Acrylamide induces dominant lethal mutations in mice following oral or intraperitoneal administration. These dominant lethal effects predominantly involve late spermatids/early spermatocytes. There is evidence that mice lacking CYP2E1 activity do not produce dominant lethal mutations when dosed with acrylamide, suggesting that metabolism to glycidamide is important with regard to dominant lethal mutations induced by acrylamide. Additional investigations for dominant lethal effect in mice pretreated with 1-aminobenzotriazole (ABT) to inhibit the metabolism of acrylamide suggest that a range of mechanisms, some of which may involve direct effects of acrylamide may be relevant to the dominant lethal effects induced in mice.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Investigations into the morphology of mouse embryos produced from matings where the males have been dosed with acrylamide are consistent with the available dominant lethal studies, although the intraperitoneal NOEL of 10 mg/kg bw/day would appear to be lower than in conventional dominant lethal studies. Studies investigating chromosomal aberrations in mice given intraperitoneal doses of acrylamide suggest that effects may occur in late spermatids, early spermatozoa and pachytene spermatocytes. There was no evidence for aneugenic effects following FISH analysis of sperm from mice dosed with acrylamide by intraperitoneal injection. However centromere positive micronuclei were observed in postmeiotic cells from matings where the males had been dosed by intraperitoneal injection with acrylamide.

Overview of genotoxicity of glycidamide

60. Members will note that the relevant studies have been summarised in Annex 1. However there has been insufficient time for the secretariat to prepare an overview and it is hoped to do this for the 12 June 2008 COM meeting.

Summary

61. The EU risk assessment report concluded that acrylamide was an *in vitro* mutagen, and *in vivo* somatic cell and germ cell mutagen. The predominant effects appeared to be clastogenic with some evidence for aneugenicity. The published evidence available since 1995 would appear to extend the effects of acrylamide to include identifiable DNA adducts and gene mutations, which are detectable in cultured mammalian cells and somatic cells *in vivo*. These effects appear to be mainly mediated by metabolism to glycidamide. The default approach recommended by COM is to assume no threshold for *in vivo* gene mutational effects unless compound specific data can be provided to support such a mechanism.

Questions for COM

62. COM is asked to consider the following questions in addition to the 16 questions on the various types of study. Members may wish to defer all or part of these questions pending the full assessment of glycidamide genotoxicity data.

i) What are the effects of acrylamide in *in vitro* genotoxicity tests. What are the mechanisms? And what role does metabolism to glycidamide play?

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

- ii) What are the effects of acrylamide in *in vivo* somatic cell genotoxicity tests. What are the mechanisms? And what role does metabolism to glycidamide play?
- ii) Does DNA adduct data affect the interpretation of the available mutagenicity data?
- iii) What are the effects of acrylamide in *in vivo* germ cell genotoxicity tests. What are the mechanisms? And what role does metabolism to glycidamide play?
- iv) What *in vivo* genotoxicity effects of acrylamide may operate through a threshold mechanism and what genotoxic effects appear to have no threshold?
- v) What research is required to resolve any questions regarding the potential for threshold genotoxic effects of acrylamide?

Secretariat January 2008

References

- Abramsson-Zetterberg L; (2003) The dose-response relationship at very low doses of acrylamide is linear in the flow cytometer-based mouse micronucleus assay, *Mutat Res* 535 (2), 215-222
- Abramsson-Zetterberg L;Wong J;Ilback NG; (2005) Acrylamide tissue distribution and genotoxic effects in a common viral infection in mice, *Toxicology* 211 (1-2), 70-76
- Adler ID;Baumgartner A;Gonda H;Friedman MA;Skerhut M; (2000) 1-Aminobenzotriazole inhibits acrylamide-induced dominant lethal effects in spermatids of male mice, *Mutagenesis* 15 (2), 133-136
- Adler ID;Gonda H;Hrabe dA;Jentsch I;Otten IS;Speicher MR; (2004) Heritable translocations induced by dermal exposure of male mice to acrylamide, *Cytogenet Genome Res* 104 (1-4), 271-276
- Backman J;Sjoholm R;Kronberg L; (2004) Characterization of the adducts formed in the reactions of glycidamide with thymidine and cytidine, *Chem Res Toxicol* 17 (12), 1652-1658
- Backman J;Kronberg L; (2007) Reaction of glycidamide with 2'-deoxyadenosine and 2'-deoxyguanosine--mechanism for the amide hydrolysis, *Nucleosides Nucleotides Nucleic Acids* 26 (2), 129-148
- Batiste-Alentorn M;Xamena N;Creus A;Marcos R; (1994) Further studies with the somatic white-ivory system of *Drosophila melanogaster*: genotoxicity testing of ten carcinogens, *Environ Mol Mutagen* 24 (2), 143-147
- Batiste-Alentorn M;Xamena N;Creus A;Marcos R; (1995) Genotoxic evaluation of ten carcinogens in the *Drosophila melanogaster* wing spot test, *Experientia* 51 (1), 73-76

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Baum M;Fauth E;Fritzen S;Herrmann A;Mertes P;Merz K;Rudolphi M;Zankl H;Eisenbrand G; (2005) Acrylamide and glycidamide: genotoxic effects in V79-cells and human blood, *Mutat Res* 580 (1-2), 61-69

Besaratinia A;Pfeifer GP; (2003) Weak yet distinct mutagenicity of acrylamide in mammalian cells, *J Natl Cancer Inst* 95 (12), 889-896

Besaratinia A;Pfeifer GP; (2004) Genotoxicity of acrylamide and glycidamide, *J Natl Cancer Inst* 96 (13), 1023-1029

Bjorge C;Brunborg G;Wiger R;Holme JA;Scholz T;Dybing E;Soderlund EJ; (1996) A comparative study of chemically induced DNA damage in isolated human and rat testicular cells, *Reprod Toxicol* 10 (6), 509-519

Blasiak J;Gloc E;Wozniak K;Czechowska A; (2004) Genotoxicity of acrylamide in human lymphocytes, *Chem Biol Interact* 149 (2-3), 137-149

Chico G;Massart C;Jin L;Vanvooren V;Caillet-Fauquet P;Andry G;Lothaire P;Dequanter D;Friedman M;Van Sande J; (2006) Acrylamide, an in vivo thyroid carcinogenic agent, induces DNA damage in rat thyroid cell lines and primary cultures, *Mol Cell Endocrinol* 257-258 (), 6-14

Davis, J. and Recio, L. (2007) Determination of a Micronuclei Frequency Peripheral Blood of B6C3F1 Mice Exposed Acrylamide for Four Weeks, ILS Report C155-01

Dobrzynska M;Lenarczyk M;Gajewski AK; (1990) Induction of dominant lethal mutations by combined X-ray-acrylamide treatment in male mice, *Mutat Res* 232 (2), 209-215

Dobrzynska MM;Gajewski AK; (2000) Induction of micronuclei in bone marrow and sperm head abnormalities after combined exposure of mice to low doses of X-rays and acrylamide, *Teratog Carcinog Mutagen* 20 (3), 133-140

Doerge DR;Young JF;McDaniel LP;Twaddle NC;Churchwell MI; (2005a) Toxicokinetics of acrylamide and glycidamide in B6C3F1 mice, *Toxicol Appl Pharmacol* 202 (3), 258-267

Doerge DR;Young JF;McDaniel LP;Twaddle NC;Churchwell MI; (2005b) Toxicokinetics of acrylamide and glycidamide in Fischer 344 rats, *Toxicol Appl Pharmacol* 208 (3), 199-209

Doerge DR;da Costa GG;McDaniel LP;Churchwell MI;Twaddle NC;Beland FA; (2005c) DNA adducts derived from administration of acrylamide and glycidamide to mice and rats, *Mutat Res* 580 (1-2), 131-141

Emmert B;Bunger J;Keuch K;Muller M;Emmert S;Hallier E;Westphal GA; (2006) Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5, *Toxicology* 228 (1), 66-76

Farmer PB;Brown K;Tompkins E;Emms VL;Jones DJ;Singh R;Phillips DH; (2005) DNA adducts: Mass spectrometry methods and future prospects, *Toxicol Appl Pharmacol* 207 (2 Suppl), 293-301

Fennell, T.R., Snyder, R.W. and Friedman, M.A. (2007) Unpublished results, (Abstract Only)

Gamboa dC;Churchwell MI;Hamilton LP;Von Tungeln LS;Beland FA;Marques MM;Doerge DR; (2003) DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice, *Chem Res Toxicol* 16 (10), 1328-1337

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Gassner P;Adler ID; (1995) Analysis of chemically induced spindle aberrations in male mouse germ cells: comparison of differential and immunofluorescent staining procedures, *Mutagenesis* 10 (3), 243-252

Gassner P;Adler ID; (1996) Induction of hypoploidy and cell cycle delay by acrylamide in somatic and germinal cells of male mice, *Mutat Res* 367 (4), 195-202

Generoso WM;Sega GA;Lockhart AM;Hughes LA;Cain KT;Cacheiro NL;Shelby MD; (1996) Dominant lethal mutations, heritable translocations, and unscheduled DNA synthesis induced in male mouse germ cells by glycidamide, a metabolite of acrylamide, *Mutat Res* 371 (3-4), 175-183

Ghanayem BI;McDaniel LP;Churchwell MI;Twaddle NC;Snyder R;Fennell TR;Doerge DR; (2005) Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts, *Toxicol Sci* 88 (2), 311-318

Ghanayem BI;Witt KL;Kissling GE;Tice RR;Recio L; (2005a) Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: evidence consistent with a glycidamide-mediated effect, *Mutat Res* 578 (1-2), 284-297

Ghanayem BI;Witt KL;El Hadri L;Hoffler U;Kissling GE;Shelby MD;Bishop JB; (2005b) Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: evidence supporting a glycidamide-mediated effect, *Biol Reprod* 72 (1), 157-163

Glatt H;Schneider H;Liu Y; (2005) V79-hCYP2E1-hSULT1A1, a cell line for the sensitive detection of genotoxic effects induced by carbohydrate pyrolysis products and other food-borne chemicals, *Mutat Res* 580 (1-2), 41-52

Holland N;Ahlborn T;Turteltaub K;Markee C;Moore D;Wyrobek AJ;Smith MT; (1999) Acrylamide causes preimplantation abnormalities in embryos and induces chromatin-adducts in male germ cells of mice, *Reprod Toxicol* 13 (3), 167-178

Husoy T;Abramsson-Zetterberg L;Olstorn HB;Paulsen JE;Alexander J; (2005) Adenomatous polyposis coli influences micronuclei induction by PhIP and acrylamide in mouse erythrocytes, *Mutat Res* 580 (1-2), 111-118

Jie YM;Jia C; (2001) Chromosomal composition of micronuclei in mouse NIH 3T3 cells treated with acrylamide, extract of *Tripterygium hypoglaucom* (level) hutch, mitomycin C and colchicine, detected by multicolor FISH with centromeric and telomeric DNA probes, *Mutagenesis* 16 (2), 145-149

Johansson F;Lundell T;Rydberg P;Erixon K;Jenssen D; (2005) Mutagenicity and DNA repair of glycidamide-induced adducts in mammalian cells, *Mutat Res* 580 (1-2), 81-89

Kjuus H;Hansteen IL;Ryberg D;Goffeng LO;Ovrebø S;Skaug V; (2005) Chromosome aberrations in tunnel workers exposed to acrylamide and N-methylolacrylamide, *Scand J Work Environ Health* 31 (4), 300-306

Klaunig JE;Kamendulis LM; (2005) Mechanisms of acrylamide induced rodent carcinogenesis, *Adv Exp Med Biol* 561 (), 49-62

Kligerman AD;Atwater AL;Bryant MF;Erexson GL;Kwanyuen P;Dearfield KL; (1991) Cytogenetic studies of ethyl acrylate using C57BL/6 mice, *Mutagenesis* 6 (2), 137-141

Koyama N;Sakamoto H;Sakuraba M;Koizumi T;Takashima Y;Hayashi M;Matsufuji H;Yamagata K;Masuda S;Kinae N;Honma M; (2006) Genotoxicity of acrylamide and glycidamide in human lymphoblastoid TK6 cells, *Mutat Res* 603 (2), 151-158

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Krebs O;Favor J; (1997) Somatic and germ cell mutagenesis in lambda lacZ transgenic mice treated with acrylamide or ethylnitrosourea, *Mutat Res* 388 (2-3), 239-248

Krishna G;Theiss JC; (1995) Concurrent analysis of cytogenetic damage in vivo: a multiple endpoint-multiple tissue approach, *Environ Mol Mutagen* 25 (4), 314-320

Lafferty, J. S., Kamendulis, L. M., Kaster, J., Jiang, J., and Klaunig, J. E. (2004) Subchronic acrylamide treatment induces a tissue-specific increase in DNA synthesis in the rat., *Toxicol Lett* 154 (1-2), 95-103

Ma XX;Yao GD;Cheng H;Zeng QL;Chen Q; (2003) [Effects of acrylamide on DNA damage in human keratinocytes], *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 21 (2), 96-98

Maniere I;Godard T;Doerge DR;Churchwell MI;Guffroy M;Laurentie M;Poul JM; (2005) DNA damage and DNA adduct formation in rat tissues following oral administration of acrylamide, *Mutat Res* 580 (1-2), 119-129

Manjanatha MG;Aidoo A;Shelton SD;Bishop ME;McDaniel LP;Lyn-Cook LE;Doerge DR; (2006) Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female Big Blue mice, *Environ Mol Mutagen* 47 (1), 6-17

Marchetti F;Lowe X;Moore DH;Bishop J;Wyrobek AJ; (1996) Paternally inherited chromosomal structural aberrations detected in mouse first-cleavage zygote metaphases by multicolour fluorescence in situ hybridization painting, *Chromosome Res* 4 (8), 604-613

Marchetti F;Lowe X;Bishop J;Wyrobek AJ; (1997) Induction of chromosomal aberrations in mouse zygotes by acrylamide treatment of male germ cells and their correlation with dominant lethality and heritable translocations, *Environ Mol Mutagen* 30 (4), 410-417

Martins C;Oliveira NG;Pingarilho M;Gamboa dC;Martins V;Marques MM;Beland FA;Churchwell MI;Doerge DR;Rueff J;Gaspar JF; (2007) Cytogenetic damage induced by acrylamide and glycidamide in mammalian cells: correlation with specific glycidamide-DNA adducts, *Toxicol Sci* 95 (2), 383-390

"Mei N; Hu J; Churchwell MI; Guo L; Moore M;Doerge DR;Chen T (2007) Genotoxic effects of acrylamide and glycidamide in mouse lymphoma cells, *Food Chem Toxicol E Pub*

Nesterova EV;Durnev AD;Seredenin SB; (1999) Verapamil contributes to the clastogenic effects of acrylamide, cyclophosphamide, and dioxidine on somatic cells of BALB/C and C57BL/6 mice, *Mutat Res* 440 (2), 171-179

Park J;Kamendulis LM;Friedman MA;Klaunig JE; (2002) Acrylamide-induced cellular transformation, *Toxicol Sci* 65 (2), 177-183

Paulsson B;Grawe J;Tornqvist M; (2002) Hemoglobin adducts and micronucleus frequencies in mouse and rat after acrylamide or N-methylolacrylamide treatment, *Mutat Res* 516 (1-2), 101-111

Paulsson B;Kotova N;Grawe J;Henderson A;Granath F;Golding B;Tornqvist M; (2003) Induction of micronuclei in mouse and rat by glycidamide, genotoxic metabolite of acrylamide, *Mutat Res* 535 (1), 15-24

Pontecorvo G; Fantaccione S (2006) Recombinogenic activity of 10 chemical compounds in male germ cells of *Drosophila melanogaster*, *Ecotox. Environ. Safe.* 65 (1), 93-101

Puppel N;Tjaden Z;Fueller F;Marko D; (2005) DNA strand breaking capacity of acrylamide and glycidamide in mammalian cells, *Mutat Res* 580 (1-2), 71-80

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Schmid TE;Xu W;Adler ID; (1999) Detection of aneuploidy by multicolor FISH in mouse sperm after in vivo treatment with acrylamide, colchicine, diazepam or thiabendazole, *Mutagenesis* 14 (2), 173-179

Schriever-Schwemmer G;Kliesch U;Adler ID; (1997) Extruded micronuclei induced by colchicine or acrylamide contain mostly lagging chromosomes identified in paintbrush smears by minor and major mouse DNA probes, *Mutagenesis* 12 (4), 201-207

Segeberback D;Calleman CJ;Schroeder JL;Costa LG;Faustman EM; (1995) Formation of N-7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA of the mouse and the rat following intraperitoneal administration of [¹⁴C]acrylamide, *Carcinogenesis* 16 (5), 1161-1165

Silvari V;Haglund J;Jenssen D;Golding BT;Ehrenberg L;Tornqvist M; (2005) Reaction-kinetic parameters of glycidamide as determinants of mutagenic potency, *Mutat Res* 580 (1-2), 91-101

Tareke E;Twaddle NC;McDaniel LP;Churchwell MI;Young JF;Doerge DR; (2006) Relationships between biomarkers of exposure and toxicokinetics in Fischer 344 rats and B6C3F1 mice administered single doses of acrylamide and glycidamide and multiple doses of acrylamide, *Toxicol Appl Pharmacol* 217 (1), 63-75

Thielen S;Baum M;Hoffmann M;Loeppky RN;Eisenbrand G; (2006) Genotoxicity of glycidamide in comparison to (+/-)-anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide and alpha-acetoxy-N-nitroso-diethanolamine in human blood and in mammalian V79-cells, *Mol Nutr Food Res* 50 (4-5), 430-436

Titenka-Holland, N., Ahlborn, Thomas, Lowe, Xiu, Shang, Nang, Smith, Marilyn T., and Wyrobek, Andrew J. (1998) Micronuclei and Developmental Abnormalities in 4-Day Mouse Embryos After Paternal treatment with Acrylamide, *Environmental and Molecular Mutagenesis* 31 (), 206-217

Twaddle NC;Churchwell MI;McDaniel LP;Doerge DR; (2004) Autoclave sterilization produces acrylamide in rodent diets: implications for toxicity testing, *J Agric Food Chem* 52 (13), 4344-4349

Tyl RW;Friedman MA; (2003) Effects of acrylamide on rodent reproductive performance, *Reprod Toxicol* 17 (1), 1-13

Vogel EW;Nivard MJ; (1993) Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination, *Mutagenesis* 8 (1), 57-81

Working PK;Bentley KS;Hurt ME;Mohr KL; (1987) Comparison of the dominant lethal effects of acrylonitrile and acrylamide in male Fischer 344 rats, *Mutagenesis* 2 (3), 215-220

Xie Q;Sun H;Liu Y;Ding X;Fu D;Liu K; (2006) Adduction of biomacromolecules with acrylamide (AA) in mice at environmental dose levels studied by accelerator mass spectrometry, *Toxicol Lett* 163 (2), 101-108

Yang HJ;Lee SH;Jin Y;Choi JH;Han CH;Lee MH; (2005) Genotoxicity and toxicological effects of acrylamide on reproductive system in male rats, *J Vet Sci* 6 (2), 103-109

Zamorano-Ponce E;Morales C;Ramos D;Sepulveda C;Cares S;Rivera P;Fernandez J;Carballo MA; (2006) Anti-genotoxic effect of Aloysia triphylla infusion against acrylamide-induced DNA damage as shown by the comet assay technique, *Mutat Res* 603 (2), 145-150