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DRAFT

MUT/08/6

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

**REVIEW OF GENOTOXICITY OF ACRYLAMIDE**

**ADDITIONAL DATA SUBMITTED MAY 2008.**

**[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 have requested a further evaluation from the COM regarding the information cited by the PPG\*. 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 ESR review completed by HSE (EU Risk Assessment report 2002) could be used as a basis for the review.

\*Polyelectrolyte Producers Group.

**Background to submission of additional data (Annex 1 submitted by PPG 9 May 2008, Annex 2 additional data from one COM member, Annex 3 overview of germ cell genotoxicity studies with acrylamide)**

[More detail on the background to the referral to COM and consideration of data on acrylamide and glycidamide are given in the accompanying paper MUT/08/07]

2. Briefly, the COM undertook a review of a submission of mutagenicity and other data on acrylamide from the PPG at the October 2007 COM meeting. A number of additional pieces of information were requested by COM members which were provided to the COM meeting on 14 February 2008 (MUT/08/01). At the February 2008 meeting, members reviewed the available genotoxicity data on acrylamide and reached a number of interim conclusions. Members specifically requested the submission of the publication by Mei N et al *Food Chem Tox*, 46, 628-636, 2008 which had only been available in abstract form at the February 2008 meeting (see additional review on glycidamide MUT/08/07). Following the 14 February 2008 meeting additional data were also sought from the authors of a number of published papers. The responses are set out below in this covering paper. One member submitted some preliminary data from *in vivo* comet assays undertaken with acrylamide (Annex 2) and members also asked for further consideration of the germ cell studies with acrylamide (Annex 3).

3. The COM were unable to commence a detailed review of glycidamide at the 14 February 2008 meeting although detailed summaries of summaries

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were presented to members as Annex 1 to MUT/08/02 . A separate review of glycidamide presenting information on the contribution of the genotoxicity data on glycidamide to the assessment of acrylamide has now been undertaken by the secretariat and is presented as MUT/08/07.

[Information on detailed summaries of studies can be found at <http://www.advisorybodies.doh.gov.uk/pdfs/mut082.pdf> (covering paper MUT/08/02.) <http://www.advisorybodies.doh.gov.uk/pdfs/mut082a1t1.pdf> (Annex 1 detailed summaries of studies) <http://www.advisorybodies.doh.gov.uk/pdfs/mut082a1t2.pdf> (short summary of all studies reviewed) <http://www.advisorybodies.doh.gov.uk/pdfs/mut082add1.pdf> (addendum summary of studies retrieved just prior to COM meeting 14 February 2008) Members will wish to note one further reference Ao et al 2008 (Mutagenesis *in press*) has been retrieved and is evaluated in MUT/08/07]

### **Additional data submitted by PPG (Annex 1 in confidence)**

4. The COM secretariat held a further meeting with PPG on 15 April 2008 to discuss the additional data submitted for COM. The minutes of this meeting are appended at the end of this draft discussion paper. A copy of the additional data submitted in May 2008 is appended as Annex 1 An overview of the additional data submitted is given below. Members will recall commenting on a proposal for further research using *Salmonella typhimurium* TA100 strains made CYP2E1 proficient. (A copy of the proposal is included as part of Annex 1) The Committee advised;

The proposed investigations by PPG focus on the apparent anomaly of the activity of glycidamide but inactivity of acrylamide in *Salmonella* and may be helpful in this respect.

However, it is unlikely the work will be of much help in resolving the key argument about thresholds with acrylamide *in vivo* in mammalian systems or whether acrylamide has several mechanisms of action or what these mechanisms may be.

5. PPG submitted two pre-publication manuscripts. These are appended as in confidence documents for members and assessors only. Zeiger E et al 2008 (prepublication manuscript) (Investigations of the Low dose Response for the induction by Acrylamide of Adducts and Micronuclei). The paper succinctly summarises many of the points raised by PPG during presentations by Dr Zeiger regarding the PPG repeat dose MN investigation in mice. The authors show evidence for saturation of CYP2E1 activation of acrylamide at relatively high dose levels.

6. Freidman M et al 2008 (submitted to a peer review journal) (Inhibition of rat testicular nuclear kinesins (krp2; KIFC5A) by acrylamide as a basis for establishing a genotoxicity threshold) presents a review the evidence for protein based targets for the genotoxicity of acrylamide. It is noted that the authors present an evaluation of the temporal association between DNA damage comet assay (in liver, bone marrow and brain) compared to DNA

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adduct data (7-GA-Gua) in liver and brain. It is concluded that DNA adduction and DNA damage may not be temporally correlated. The authors also refer to Jenkins et al (Mutagenesis, 2005, 20, 389-393), and Swenburg et al (Env Health Perspect, 1985, 62, 177-183) in support of evidence for DNA repair of N-7 and N-3 alkylation of Guanine. This is a similar argument to that previously presented to COM. No acrylamide specific data on repair of DNA adducts are available.

### **Additional data submitted by one COM member (Annex 2 in confidence)**

7. One member has supplied some initial data from the JaCVAM interlaboratory trial with the in-vivo comet assay. The preliminary data indicates positive results for acrylamide in liver, stomach, and kidney. No further details are available. The data support a conclusion that acrylamide induces multi tissue DNA damage in rodents.

### **Responses from authors**

8. At the February 2008 meeting, members asked for clarification on certain aspects of some of the references that were included in the systematic review. The Secretariat has undertaken to contact the authors of these references to seek clarification

**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**

9. The secretariat asked Dr Besaratinia whether he had any generated any data on the metabolic competency of the Big Blue mouse embryonic fibroblasts. Also, clarification on the timing of the experiments was also sought to address the point raised by Prof. Zeiger of the Polyacrylamide Producers Group (PPG).

10. Dr Besaratinia replied: "We have not quantified the metabolic capacity of Big Blue MEF to convert acrylamide to glycidamide. The two studies were conducted independently using the same batch of 1<sup>st</sup> passage MEF. In the 2<sup>nd</sup> study, we re-examined the cII mutant frequency induced by acrylamide alongside that of glycidamide vs control. The mutant frequency data on acrylamide and control were virtually identical to those we'd reported earlier in our 1<sup>st</sup> study. For this reason, and of course, due to time & resource constraints, we decided not to re-establish the mutation spectrum induced by acrylamide."

**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**

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11. The secretariat asked Dr Doerge (the corresponding author) for more information regarding the N1-GA-dA adducts, asking whether it was correct that methodological limitations prevented this adduct from being detected, rather than demonstrating that this adduct is not formed in vivo. Dr Doerge replied: *“Yes, the highly polar nature of this adduct made it impossible to sort it out from the background of interferences, even with the added step of converting it to the less polar N6-adduct via base-catalyzed rearrangement. While we could have gone back and worked harder to solve this technical issue, we chose not to as described below.”*

12. The secretariat went on to ask for clarification on the ratios of three adducts. The Gamboa da Costa paper indicates that in vitro the ratio of the N7-GA-Gua : N1-GA-dA : N3-GA-Ade is 74:16:1. Since the ~70:1 ratio for the N7-GA-Gua : N3-GA-Ade adducts seems to also be apparent in vivo; is it plausible that the ratio of N7-GA-Gua to N1-GA-dA adducts may also occur at a 5:1 ratio in vivo, yet not be detected?

13. Dr Doerge replied: *“Yes, I do think that the in vitro data do accurately reflect those in vivo because it is just the reflection of DNA reactivity with glycidamide, which we have also shown to be widely distributed to all tissues and to form N7-GA-Gua and N3-GA-Ade in roughly the same ratios everywhere.”*

14. *“The other result to consider is our mutagenesis assay in Big Blue mice where the major point mutations in the cII transgene were sequenced (Manjanatha et al., 2006). There was no evidence for mutations at A and in fact the major mutants were GC --> TA transversions. This finding argues against the importance of the N1-GA-dA adduct while supporting formation the major adduct, N7-GA-Gua, as pivotal in mutagenesis by acrylamide.”*

15. In response to this, the Secretariat asked for clarification on the evidence for no mutations at A in the Manjanatha Big Blue mouse study; since the paper says that, although G to T transversions predominate, A to G transitions and A to C/T transversions make up 5-10% of the total, and this could be taken this to mean this study shows mutations consistent with N3 and possibly N1 dA adducts. Dr Doerge agreed that this was a plausible hypothesis.

**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**

16. Dr Sun (the corresponding author) was asked to clarify the units (ng/g) used in Figure 2 of the paper. The materials and methods section reports that this is nanograms of acrylamide equivalents per gram of tissue. Dr Sun was asked whether ‘tissue’ relates to isolated DNA, protamine, haemoglobin and serum albumin; or do you mean wet weight of the tissue before processing. Dr Su was also asked whether 1 ng/g represents the limit of detection for this method, since this seems to be limit of Figure 2 measures adducts down to

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approximately 1 ng/g. Does this represent the limit of quantification for this method?

17. Dr Sun replied: "By 'tissue', we mean the dry weight of the isolated DNA, protamine, haemoglobin and serum albumin. Actually, the data in Figure 2 shows the adducts down to approximately 0.1 ng/g rather than 1 ng/g. The limit of quantification of our experiment should be acquired by the data in Figure 2, namely around 0.1 ng/g. However, this doesn't represent the limit of quantification for this method. If we have the labeled acrylamide with higher specific activity, the limit should be even lower. "

### **Consideration of germ cell genotoxicity of acrylamide**

18. The Committee agreed to further consider germ cell toxicity at the 12 June 2008 meeting. The draft conclusion abstracted from MUT/08/02 is given below for members evaluation. The draft discussion evaluation is appended as Annex 3 for ease of reference.

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

19. 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. 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.

### **COM questions**

20. Members are asked to comment on the additional data provided by PPG.
21. Members are asked to comment on the comet assay data submitted by one COM member

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22. Members are asked to comment on the responses from authors
23. Members are asked to finalise conclusions on the germ cell genotoxicity of acrylamide.

**Secretariat May 2008**

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

### REVIEW OF ACRYLAMIDE: MEETING WITH POLYELECTROLYTE PRODUCERS GROUP (PPG): 15 APRIL 2008

#### Present

Dr D Marroni	President PPG
Dr M Friedman	Consultant
Mr J Battershill	HPA COM Secretariat.
Dr D Mason	FSA COM Secretariat.

#### Item 1: Introduction

1. The COM Secretariat thanked attendees for coming to the meeting and informed attendees that the purpose was to outline the procedures for completing the COM review, to request submission of any outstanding data from PPG and to discuss potential areas for further research. PPG thanked the secretariat for the opportunity to discuss the completion of the COM review.

#### Item 2: Discussion of topics

##### Procedures

2. The Secretariat noted that PPG had made two oral presentations to the COM which had fully covered PPG's evaluation of the genotoxicity of acrylamide and glycidamide. PPG agreed this comment. The Secretariat considered that it would be useful to ask PPG to submit any remaining information for consideration at the 12 June 2008 COM meeting. It was hoped to identify key areas for further submission during this meeting. The Secretariat reported that an update on PPG data and a review of glycidamide genotoxicity data would be presented to the COM on the 12 June 2008. PPG could attend the meeting as 'Observers'. The Chairman could ask for clarification of any points during the COM discussion. A draft working paper would be produced based on the COM discussions summarising all the genotoxicity data. This could either be circulated to members after the June 2008 meeting or submitted to the October 2008 meeting. The draft working paper would be placed on the COM internet site and PPG would have an opportunity to comment. The Secretariat reported that, subject to agreement from COM, they would endeavour to finalise the review by the end of 2008.

##### Submission of additional data

3. PPG noted the difference of interpretation regarding the dose-response evaluation of the PPG repeat dose MN study in mice between COM and Dr Haseman and asked what further representation could be made on this topic. The secretariat noted the committee's view that in the event that several models could equally explain the dose-response data from this study, the preference was to adopt the simplest approach regarding the evaluation of the dose-response which in this instance was a linear-dose response. The

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secretariat noted the limitations of the MN assay regarding sensitivity and noted COM views that further consideration of the dose –response data from this study using the historical control data instead of the concurrent control data would be unlikely to change the COM evaluation of the study.

4. It was suggested further consideration should focus on mechanisms of genotoxicity with regard to identifying potential threshold mechanisms. In this regard the secretariat considered the COM had accepted a number of mechanisms could be responsible for the genotoxicity of acrylamide and glycidamide and that some mechanisms such as oxidative DNA damage and inhibition of kinesin proteins were likely to be threshold phenomena. However, these mechanisms are not mutually exclusive and it was suggested that further consideration would need to focus on the metabolism of acrylamide to glycidamide, reaction of glycidamide with DNA to form adducts, and subsequent conversion of DNA adducts to mutations. The secretariat provided a draft diagram of potential mechanisms, which would be finalised and submitted to COM. The diagram would be useful to aid in the discussion of the mechanisms of acrylamide and glycidamide genotoxicity. Potential mechanisms for a threshold included rapid detoxication of glycidamide and repair of DNA adducts. The Secretariat recommended that any further submission present compound specific data on potential mechanisms for a threshold for DNA adduction and repair. It was agreed that any further submission should consider somatic cell and germ cell genotoxicity. The Secretariat noted that one key topic for consideration was the potential for repair of DNA adducts formed from acrylamide and glycidamide for both somatic cell and germ cell genotoxicity.

5. PPG noted that they had recently made a submission of data to the EPA which might also be valuable for COM consideration.

6. The Secretariat noted that one COM member has recently forwarded some *in vivo* comet data on acrylamide. The study had been part of the JaCVAM OECD guideline initiative for the comet assay which had reported positive results for acrylamide in a wide range of tissues. The study had not been specifically designed to investigate dose-response at low dose levels. The Secretariat had asked for access to the full study report.

7. The Secretariat outlined the initial responses from a number of research groups to questions raised by the COM. The agreed response from these authors would be provided to the COM for the 12 June 2008 meeting. There was discussion on the approaches to the detection of glycidamide adducts with adenine. PPG expressed the view that the N1-GA-Ade could be detected and levels measured.

### Further research

8. The Secretariat noted the recent submission from PPG for a proposal to investigate DNA reactive mechanism for mutations in *Salmonella typhimurium* TA 100 (containing YG7108 metabolically competent for CYP2E1) with acrylamide. COM members had received the PPG document by postal circulation and had agreed the proposal would be helpful investigation regarding whether glycidamide was formed from acrylamide in these bacteria and the potential mutagenicity under these test conditions. However the proposed study would not provide an insight into potential mechanisms for threshold for DNA reactivity and mutagenicity of glycidamide.

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9. PPG noted these comments and reported that they had considered a step wise approach to the investigation of mechanisms of acrylamide mutagenicity was appropriate and proposed to follow up the studies in *Salmonella* with studies in mammalian systems such as the mouse Big Blue fibroblasts. This would eventually provide comprehensive data on the metabolism of acrylamide to glycidamide in all relevant tests systems along with formation of DNA adducts and mutations. PPG agreed to resubmit the proposal with additional information on further tests which were envisaged.

10. The Secretariat noted the published evidence previously seen by COM which indicated the efficiency of conversion of acrylamide to glycidamide in rodents was greater at low doses compared to high doses. It was suggested that any further proposal or outline for further research should include a strategy to investigate the potential for threshold for DNA adduction and subsequent mutations due to acrylamide and glycidamide.

11. PPG noted the most recent investigations using transcriptomic approaches to investigate gene expression changes in response to exposure to acrylamide in a number of test systems and suggested the lack of a DNA repair response might be suggestive of a threshold for exposure to acrylamide. PPG noted Dr JH Van Delft (Maastricht University) had published information on a potential transcriptomic pattern for genotoxic carcinogens compared to non-genotoxic carcinogens. (Van Delft J H M *et al. Carcinogenesis* , **25**: 1265-1276, 2004.). PPG considered this to be a potential area for further research with acrylamide.

12. PPG noted a further area of investigation would be to investigate the correlation of tissue specific DNA adduction and mutation with the observer target organ carcinogenicity seen in the rat. They noted that a study of acrylamide mutagenicity in transgenic Big Blue rats was underway and data would be available in the near future. The Secretariat noted that there was no referral to the COC for evaluation of carcinogenicity. The key area of evaluation was the evaluation of mechanisms of acrylamide induced mutations in transgenic mice (Majanatha MG *et al Environ Mol Mut*, **47**, 6-17, 2006). The available data suggested that acrylamide was metabolised to glycidamide with a DNA reactive mechanism for the observed increase in mutation frequency in transgenic mice.

### **Item 3: Concluding Comments**

13. The Secretariat asked PPG to submit all additional data by 5/6 May 2008 in order to meet deadlines for the 12 June 2008 COM meeting. The Secretariat asked for research proposals and submitted evaluations to be compound specific for acrylamide and glycidamide. The Secretariat noted EFSA's 11th Scientific Colloquium - Acrylamide carcinogenicity - New evidence in relation to dietary exposure - 22 and 23 May 2008, Tabiano (PR), Italy. PPG reported they would be attending this meeting.

**J.Battershill**  
**For HPA COM secretariat.**  
**30 April 2008**