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MUT/08/1

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

REVIEW OF GENOTOXICITY OF ACRYLAMIDE

SUBMISSION FROM THE POLYELECTROLYTE PRODUCERS GROUP (PPG): ADDITIONAL DATA SUBMITTED DECEMBER 2007 AND JANUARY 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 14 December 2007. Annex 2 submitted 15 January 2008)

2. 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 included; additional historical control data regarding the oral 28 day rat MN assay undertaken by PPG, a copy of a paper by Healy L et al (Mutagenesis, 16, 163-168, 2001) which reported on evidence for a time-dependent accumulation of MN in peripheral blood of mice exposed to benzene, a copy of the studies by Witt KL and colleagues (Mutation Research, 2007) which presented an oral dose response evaluation for acrylamide induction of MN in bone marrow and peripheral blood of rats and mice, and additional information on in-vivo transgenic mutation assays undertaken with acrylamide (from Lambert IB et al Mutation Research, (2005), 590, 1-280.)

3. The COM secretariat held an initial meeting with PPG on 14 December 2007 to discuss the additional data submitted for COM. The minutes of this meeting are appended to this draft discussion paper. A copy of the additional data submitted in December 2007 is appended as Annex 1 and additional information submitted in January 2008 is appended as Annex 2. An overview of the additional data submitted is given below.

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Additional data from PPG

Historical control data from PPG repeat dose MN study

4. Relevant information for MN-NCE are given on the first page of the additional data submitted by PPG in December 2007 (Annex 1). The Secretariat asked on 14 December 2007 for relevant information for MN-PCEs to be reported and for an interpretation of the dose-response data from this study based on all available information.

5. Additional plots of historical control data have been provided in Annex 2 along with a scatter diagram of MN-NCE incidence in the low dose range. Only one animal was not within 2 SDs of the historical control mean. PPG have provided a graph of MN response and DNA adducts based on the results from the PPG study. It is noted Dr Haseman may be able to provide more comments on these data at the February 2008 meeting. PPG have also provided a copy of a review by Professor Swenburg which considers the dose-response for exposure and effect of genotoxicants. At low dose, it is proposed that endogenous DNA damage predominates.

6. Do Members have any additional comments on the dose-response data for acrylamide induction of micronuclei *in-vivo* and additional data submitted by PPG. Can the COM reach a conclusion on the available data?

Information on benzene MN induction with regard to accumulation of MN in repeat dose studies.

7. The publication cited at the October 2007 meeting (Healy et al 2001) has been appended in Annex 1. However PPG note the observed ratio of MN-PCE to MN-NCE from acrylamide dose animals and evidence for no substantial difference in response to orally administered acrylamide at 5 or 28 days support the view that there is no accumulation of MN formation in rodents following repeated dosing with acrylamide. Some additional comments have been provided in the covering e-mail to Annex 2.

8. Do Members have any additional comments on this aspect of the *in-vivo* mutagenicity of acrylamide?

A copy of the Witt et al study on acrylamide

9. Copies of the prepublication and final publication papers are appended in Annex 1. The objective of citing this publication is to note the comparatively similar dose-response for MN induction in mice dosed with acrylamide between testing laboratories (PPG, Abramasson-Zetterberg Mutat Res, 535, 215-222, 2003 and Witt et al 2007). The data for acrylamide dosing in rats is noted, which would be included in the secretariat review of mutagenicity of acrylamide. This study was funded by NIEHS (Annex 2)

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[A interesting generic observation from the Witt paper is the evidence to support comparatively similar data for MN induction are recorded when using flow cytometric and manual methods of counting MN for a number of in-vivo mutagens including acrylamide.]

10. Do Members have any additional comments on this study with respect to the evaluation of acrylamide?

Further analyses of MNrets/MNNCEs with regard to dosimeters such as N7GAGua.

11. PPG informed the COM secretariat at the meeting on 14 December 2007 that additional analyses were currently being undertaken. PPG note |Dr Haseman may be able to provide additional comments at the February 2008 COM meeting.

Evaluation of in-vitro and in-vivo transgenic mutation assays

12. The COM noted additional studies might be available. Information contained in the publication by Lambert I et al (Mutat Res, (2005), 590, 1-280) suggests that a number of *in-vivo* transgenic mutation studies were conducted by Health Canada (extract from Appendix A pages 124-125 from the Lambert et al publication appended as part of Annex 1 to this discussion paper). The secretariat and PPG are attempting to gain access to the full study reports. Some additional data on these studies has been tabulated in Annex 2 which report more details of conduct and results of studies. The results of the studies undertaken for Health Canada (reported by Dr Douglas) show evidence for a positive effect in bone marrow which was not identified in a repeat study.

13. Dr Zeiger has provided an additional evaluation of the transgenic mutation studies. Some information on the possible role of oxidative stress in the observed responses for genotoxins is provided. An evaluation of the response to acrylamide using the *hprt* and *cII* genes is provided. A number of comments on the conduct of the studies by Besaratinia 2003, 2004 are provided. Members will wish to consider this submission when reviewing these studies. (Papers cite in support of the submission from Dr Zeiger have been provided but have not been forwarded to members at this stage of the review).

14. Do Members have any additional comments to make on the available information provided by PPG on *in-vitro* and *in-vivo* transgenic mutation assays?

Additional paper on testicular effects of acrylamide in rats

15. A copy of a prepublication report by Dr Friedman and colleagues is appended in Annex 2 which summarises the main comments made regarding the potential involvement of acrylamide inhibition of kinesins in the genotoxicity of acrylamide.

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COM questions

16. Members are asked to comment on the additional data provided by PPG.

Secretariat January 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): 14 DECEMBER 2007

Present

Dr D Marroni
Dr M Friedman

President PPG
Consultant

Mr J Battershill
Dr D Mason

HPA COM Secretariat.
FSA COM Secretariat.

Mr K Mistry

DH Administrative support for COM secretariat.

Item 1: Introduction

1. The COM Secretariat thanked attendees for coming to the meeting and informed attendees that the purpose was to review the data submitted by PPG on the 6 December 2007 which addressed the questions and request for further data requested by the COM at the October 2007 meeting. PPG thanked the secretariat for the opportunity to present additional data and reported they were happy with the technical debate at the October 2007 meeting.

2. The Secretariat outlined questions arising from the additional data submission in the order set out by PPG and noted that some additional questions had arisen through the ongoing review of published studies on acrylamide and glycidamide which required reference to the PPG submission and presentation to the COM.

Historical control data on MN formation erythrocytes in mice.

3. The secretariat noted the data on MN-NCE and requested data for MN-RET. PPG were asked to interpret the dose-response data in the light of submitted background data on historical control information. It was noted that additional dose-response data for HB adducts might be helpful particularly with regard to the validity of such adducts as dosimeter of exposure. PPG noted that the statistical evaluation of the dose response data might need additional input from Dr Haseman (NIEHS, USA). There was discussion of the possible influence of low levels of acrylamide in diet as a possible source of HB-adducts reported in control animals. PPG agreed to look at these data in details and report any new information.

Information from Healy (Mutagenesis, 16, 163-8, 2001)

4. The secretariat noted that this paper had been submitted to support the accumulation of MN in studies using repeated exposure regimes to benzene. However the time course studies were not directly related to the acrylamide

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study from PPG which had used one sampling time point after 28 days. PPG explained that the evaluation of the 28 days repeat dose MN assay in mice referred to the ratio of MN-RET to MN-NCE where the ratio should approach 1 if there was evidence for accumulation of MN formation. PPG were asked to present this proposal in detail using appropriate examples and referenced studies. PPG also noted that there was comparatively little difference between oral 5d and 28d response to acrylamide at similar dose levels thus supporting little evidence for accumulation of MN.

Data from Witt et al (Mutation Research, 11 Aug 2007 E-pub).

5. The secretariat asked for confirmation that the study had been published and for information on the source of funding. PPG confirmed a publication date (see above) and that funding had been by NIEHS. PPG confirmed that the purpose of evaluating the Witt study was to compare responses, rather than a detailed dose-response analyses of these data. The secretariat noted that the PPG presentation had also included data on MN-NCEs from the Witt study, which were not reported in the publication and that the data for the rat study looked equivocal. PPG agreed to obtain appropriate information from the study authors.

Further evaluation of MN and N7-GA-Gua data.

6. PPG noted that they were investigating the feasibility of additional experiments to measure DNA adduct formation in bacteria and would report on progress in due course. The secretariat asked if further analysis of the data presented to COM at October 2007 would be forthcoming. PPG indicated that additional analyses were being undertaken by Dr Haseman.

Evaluation of response in Big Blue mouse

7. The secretariat asked for detailed responses supported by reference statements for the evaluation of cII response at GGGG repeats (considered by PPG to be small deletions) and the evaluation of the HPRT response in Big Blue mice (considered to be possibly deletions/rearrangements) The secretariat noted these would be important for the evaluation of these data. The summary review to date produced by the secretariat indicated significant increases in mutation frequency, and for cII changes in mutation spectra with acrylamide and glycidamide consistent with a positive response to a point mutagen. The secretariat noted they would also refer to the PPG submission to the October 2007 meeting.

8. In addition it was noted that other transgenic mutation studies were reported in a paper by Lambert I (Mut Res, 590, 1-280, 2005). The unpublished studies were believed to be sourced from Health Canada. PPG and the secretariat agreed to independently try to seek these data. PPG reported they would also seek whether there were any other transgenic rodent studies available which were not identifiable in the published literature.

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9. Overall the secretariat noted that Majantha (Env Mol Mutagen, 47, 6-17, 2006) would be key and that the COM would need to consider the evidence from this study carefully.

Correlation between end points

10. The secretariat queried if aneugenicity was always reported at higher doses than clastogenicity and whether DNA adducts were dose-related through the origin. PPG considered that Dr Haseman might be able to comment on these topics at the February 2008 meeting. A key question was the conversion of DNA adducts to mutations and the dose-response for mutagenicity. The secretariat noted that the in-vivo DNA adduct studies had not been assessed at this juncture but would be before the COM meeting in February 2008. PPG noted that additional plotting of dose-response had been undertaken by Dr Swenberg and agreed to provide these data for the COM.

11. PPG noted that a review of time course for alkylation compared to DNA repair was in the process of being drafted. A short overview would be made available for the COM. One possible argument was that oxidative DNA damage was responsible for most DNA damage in vivo and that alkylation proceeded beyond the repair of DNA damage.

Additional questions from COM members post October 2007 meeting

12. The secretariat thanked PPG for the detailed response to additional questions. From the review of published papers to date, the secretariat noted Besaratina 2003 (J Natl Cancer Inst, 95, 889-896, 2003) indicated that there was a gene mutation effect in Big Blue mouse embryo fibroblasts exposed to acrylamide. The secretariat described a dose-response assessment of mutagenicity and cytotoxicity which had been undertaken by the secretariat recently. One possible explanation was that the mutagenicity of acrylamide in this test system reduced as cytotoxicity affected the metabolism of acrylamide to glycidamide. PPG agreed to consider this paper and comment.

Concluding comments

13. The secretariat noted the review of published papers was approximately 75% complete and that these summaries would be used to complete a draft discussion cover paper. PPG asked if any questions from Com members could be directed to Dr Haseman before the COM meeting in February 2008. The secretariat agreed to contact relevant COM members for comments on this aspect. PPG noted that additional reviews of role of metabolism were undergoing (predominantly for neurotoxicity) and this work might have relevance to the COM review. PPG asked that mutagenic potency be considered. PPG asked about FSA position regarding the recently published paper on cancer epidemiology (ref...). It was explained that the current risk assessment approach considered no threshold for carcinogenicity and the risk management approach was therefore to reduce exposure to as low as reasonably practical. PPG asked if COC were to consider acrylamide. The secretariat considered this would need to be considered in due course with reference to conclusions reached by COM.

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J.Battershill
For COM secretariat
14 December 2007.