

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

Review of the Genotoxicity of Acrylamide

EU Risk Assessment Summary and Overview of the COM Review Strategy

[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 (MUT/07/16 Annex 2). 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 2002 EU Risk Assessment Report (ESR), drafted by HSE and agreed by an EU technical Group, 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[†]. 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.

Introduction to COM review

3. Discussion paper MUT/07/16 presents an overview of the submission from PPG. Discussion paper MUT/07/17 presents an overview of the EU risk assessment report and the strategy being used by the secretariat to complete the review of the genotoxicity of acrylamide.

Advice requested from COM

4. The COM is asked to evaluate the summary of the EU risk assessment report, and comment on the strategy employed for the COM review on genotoxicity data published subsequent to the EU risk assessment report.

[†] <http://www.advisorybodies.doh.gov.uk/com/acryla.htm> (MUT/07/16, Annex 1)

EU Risk Assessment Report summary

5. The EU Risk Assessment Report was prepared by the HSE, acting as rapporteur to the European Union. The evaluation was carried out in accordance with Council Regulation (EEC) 793/931 on the evaluation and control of the risks of “existing” substances. The risk assessment was published in 2002, but was based on a literature review performed in 1995.

6. Annex A contains a tabulated summary, prepared by the Secretariat, of the genotoxicity data reviewed in the EU Risk Assessment Report. Annex B contains the full genotoxicity section from the report. The report concludes:

“Although acrylamide is not mutagenic in bacteria, its mutagenic potential is clearly shown in mammalian systems in vitro. It is a direct-acting mutagen and there is also a large body of evidence clearly demonstrating that acrylamide is genotoxic in vivo to both somatic cells and germ cells. In the case of germ cells, acrylamide has been demonstrated to induce heritable mutations.”

7. The evidence supporting this conclusion can be summarised thus:

In vitro

Bacterial gene mutation: generally negative in the presence and absence of metabolic activation.

Mammalian gene mutation: a mixture of positive and negative results in the presence and absence of metabolic activation. EU Risk Assessment Report concludes acrylamide is a direct acting mutagen.

Chromosomal aberrations: positive in the presence and absence of metabolic activation. EU Risk Assessment Report concludes acrylamide is a direct acting clastogen.

UDS: a mixture of positive and negative results in cultured rat hepatocytes. EU Risk Assessment Report finds the data inconsistent.

Other *in vitro* studies: positive results in sister chromatid exchange and cell transformation assays.

In vivo

Chromosomal aberrations: a mixture of positive and negative results in mouse bone marrow.

Micronucleus: generally positive, inducing micronuclei in bone marrow and spleen.

UDS: negative in one available study.

Gene mutation: positive results in a mammalian spot test and a LacZ transgenic mutation assay.

DNA adducts: evidence for protein and DNA binding of ¹⁴C-acrylamide.

In vivo germ cell

Chromosomal aberrations: a mixture of positive and negative results in one cell zygotes and spermatids.

Micronucleus: positive in all (3) assays.

UDS: positive in all (2) assays.

Dominant lethal: positive in all (6) assays.

Heritable translocation: positive in all (2) assays.

Specific locus: positive in all (2) assays.

Transgenic mice: negative when testicular preparations were examined for LacZ mutations.

DNA Damage: increased single strand breaks in mice following i.p. exposure.

Questions asked of the Committee

8. The Committee is asked to consider the following questions:
 - i. Do Members agree with the EU Risk Assessment Report conclusion (see paragraph 6)?
 - ii. Would Members comment on whether it is possible that there may be some metabolic activation of acrylamide in mammalian cell assays?
 - iii. Are the results of the LacZ transgenic mutation assay consistent with a positive result (The EU Risk Assessment Report considered “the full significance of the unvalidated assay was unclear”)?
 - iv. Are Members still content to use the EU Risk Assessment Report as a basis for their review?

COM Review – Literature Searching Strategy

9. In order to find data published after the EU Risk Assessment Report literature search, the Secretariat has conducted a search of the scientific literature using Reference Manager™ (version 10) to consult the PubMed database[‡] using the following search term:

(acrylamide OR glycidamide) AND ("DNA damage" OR "DNA adduct" OR aneugen OR clastogen* OR genotoxic* OR mutagen*) AND 1995:2007 [pdat]*

10. This search identified 235 papers. These were then sifted to exclude papers where the abstract did not indicate a primary report of genotoxicity data. Foreign language papers which did not provide a title and abstract in English were also excluded. Of the 235 papers identified by the search, 172 were excluded. Annex C lists the reasons for excluding the various papers; the majority of the exclusions were structural biochemistry papers using acrylamide to quench the fluorescence of solvent accessible tryptophan residues.

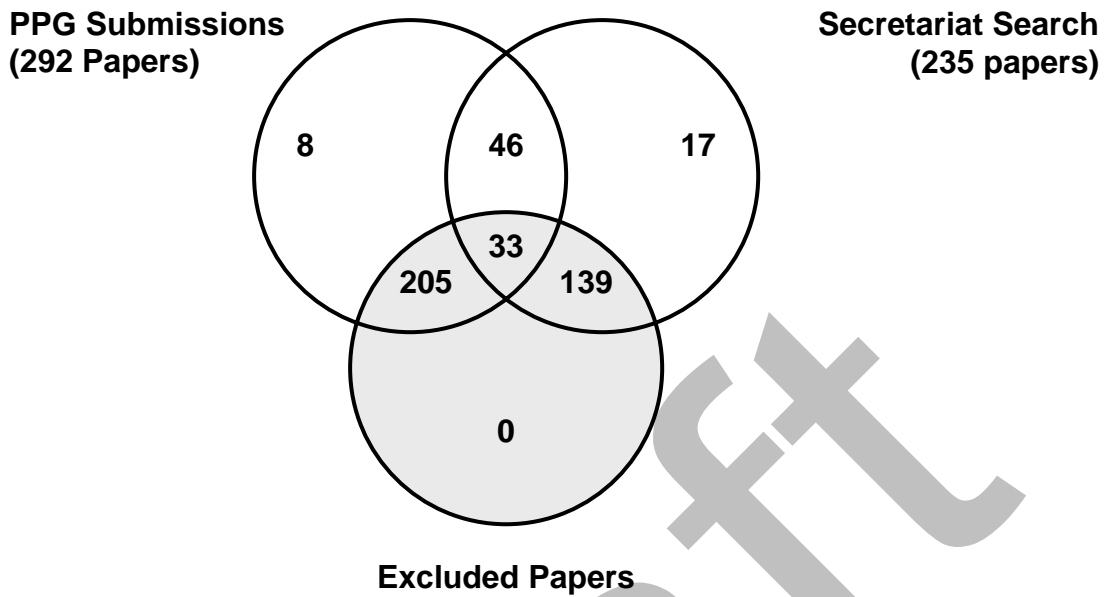
11. The Secretariat is currently reviewing the remaining 63 papers that satisfy the inclusion criteria. These will be summarised and the results tabulated, ready for the next COM meeting.

12. In total, the PPG has submitted 292 references for consideration. The Secretariat has sifted these to exclude references where the title did not indicate a primary report of genotoxicity data. When the title was ambiguous, the abstract was examined on the same criteria. As before, foreign language references which did not provide a title and abstract in English were excluded. Of the 292 submitted references, 238 were excluded. Annex D lists the reasons for excluding the various references. The majority of the exclusions were references presenting non-genotoxicity studies, predominantly neurotoxicity studies.

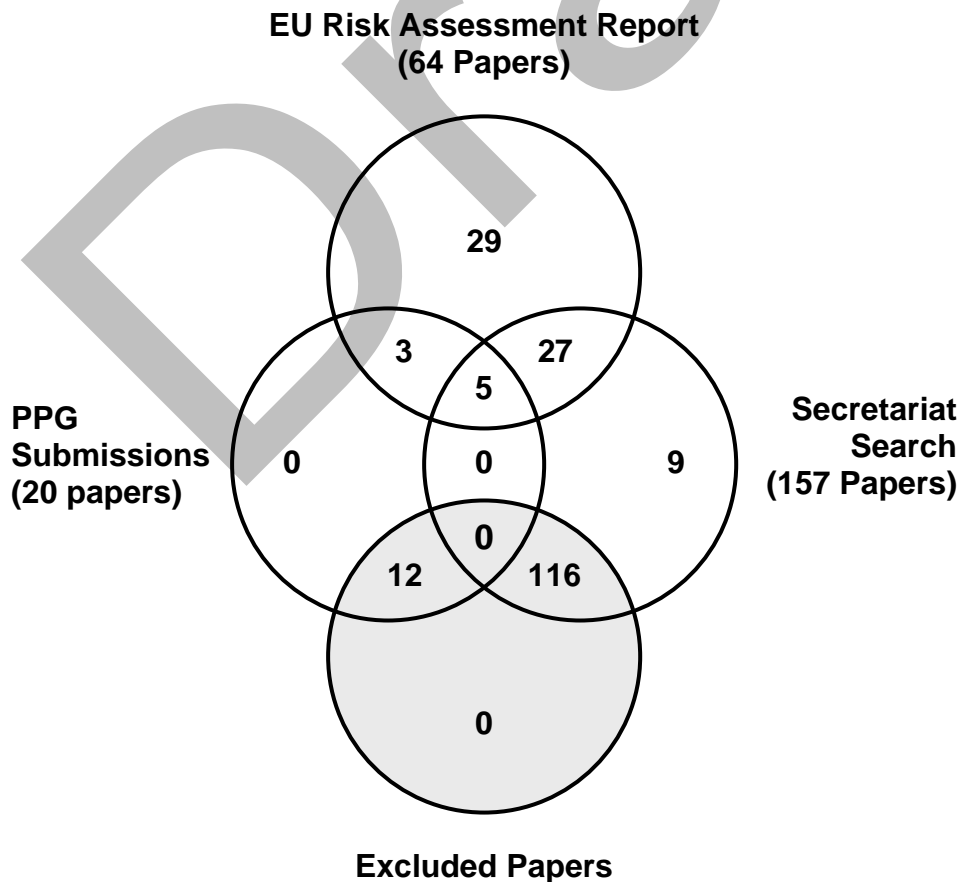
13. There were 54 references which met the inclusion criteria. Of these, 46 were also identified in the Secretariat's search. The PPG submissions contained an additional 8 potentially relevant references. These included a poster presentation on germ cell toxicity, a UDS assay, an embryo micronucleus study and a dominant lethal study. There were also 4 unpublished studies, including one micronucleus assay and one dominant lethal study. If these 8 references are available and are found to be relevant; they will be summarised, the results tabulated, and included in the COM review which will be presented at the next meeting.

[‡] <http://www.pubmed.gov>

14. The overlap of the PPG submitted papers and the search performed by the Secretariat is shown in the Venn diagram below:



15. The same search criteria were used to search prior to 1995 (Further Details in Annex E). This was compared with the PPG Submissions and the EU Risk Assessment Report in the Venn Diagram Below:



16. This search identified an additional 9 papers which were included in neither the EU Risk Assessment Report, nor the PPG Submissions. These are listed in Annex E.

Additional Literature Searches

17. Subsequent to the PubMed search, searches of additional databases have been commissioned. The details of these searches are laid out in Annex F. They identified 151 additional references published after 1995, of which 4 met the inclusion criteria and will be considered for the COM review.

Questions asked of the Committee

18. The Committee is asked to comment on the search strategy and consider the following questions:

- i. Are Members content with the search criteria?
- ii. Are Members content with the exclusion criteria?
- iii. Should the additional studies published prior to 1995 be included in the COM review?
- iv. Is the COM content with the proposal to produce a full statement on the genotoxicity of acrylamide?

Secretariat August 2007

Annexes

- Annex A: Summary of EU Risk Assessment Report Genotoxicity section 4.1.2.7.
- Annex B: EU Risk Assessment Report Genotoxicity section 4.1.2.7, pages 140-152
- Annex C: Reasons for excluding papers from the Secretariat search
- Annex D: Reasons for excluding papers from the reference lists submitted by PPG
- Annex E: The results of a search for genotoxicity papers published prior to 1995
- Annex F: The results of searches of additional databases for genotoxicity papers published after 1995.

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Summary of the Genotoxicity Data Presented in the EU Risk Assessment Report

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Review of the Genotoxicity of Acrylamide

Mutagenicity Section 4.1.2.7 from the EU Risk Assessment Report (ISBN 92-894-1250-X)

This document will not be included on the COM website, but may be freely downloaded from the following address:

http://ecb.jrc.it/documents/Existing-Chemicals/RISK_ASSESSMENT/REPORT/acrylamidereport011.pdf

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

Review of the Genotoxicity of Acrylamide

Secretariat Acrylamide Search Exclusions

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The following search identified 235 papers:

(acrylamide OR glycidamide) AND ("DNA damage" OR "DNA adduct" OR aneugen OR clastogen* OR genotoxic* OR mutagen*) AND 1995:2007 [pdat]*

These were sorted according to the following inclusion criteria:

- Abstract should indicate a primary report of mutagenicity data
- A title and abstract should be available in English.
- Where the article is foreign but an English abstract is provided, the abstract should indicate study type and result.

Of the 235 papers identified in the search, 172 were excluded for the following reasons:

Non mutagenicity studies testing acrylamide:

- 1 Carcinogenicity study
- 1 Epidemiology study
- 3 Food surveys
- 1 Neurotoxicity study
- 4 Other toxicity studies
- 1 Rainbow trout micronucleus study (not considered a relevant species)
- 18 Toxicokinetics studies
- 1 Mechanistic study looking at microtubules

Reviews on acrylamide

- 21 Reviews relating to genetic toxicology of acrylamide

Modelling associated with acrylamide

- 1 Cancer risk
- 1 Genotoxicity
- 1 Mechanism based modelling of cancer risk based on macromolecule adducts.

Papers using methods requiring acrylamide:

- 74 Protein structure experiments using fluorescence quenching
- 6 Protein Electrophoresis
- 8 Other Methods: Acrylamide used as an electron scavenger; Acrylamide derivatisation; Experiments using polyacrylamide matrices; Substrate for bacterial aliphatic amidases;

Papers mentioning other chemicals with 'acrylamide' in the name:

- 18 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide
- 1 Acrylonitrile
- 1 Acrylamide Copolymers
- 2 Papers on receptor inhibitor with an acrylamide functional group

Others:

- 3 Articles in a foreign language; 2 provided no abstract in English, and 1 provided an English abstract but mentioned genotoxicity testing, but gave no details of what the testing was or the result.
- 4 Papers mentioning acrylamide in the keywords but not the abstract

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Excluded papers from the PPG submission, reference list and additional e-mailed references

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These were sorted according to the following inclusion criteria:

- Title should indicate a primary report of mutagenicity data
- Where the title provides insufficient information, the abstract should indicate a primary report of mutagenicity data.
- The title and abstract should be provided in English

Of the 292 papers identified in the search, 238 were excluded for the following reasons:

Non mutagenicity studies testing acrylamide:

- 18 Carcinogenicity or related studies
- 6 Developmental or reproductive toxicity studies
- 53 Neurotoxicity or related studies
- 21 Other Toxicity studies
- 8 Mechanistic studies (including cytoskeletal)
- 37 Toxicokinetic, metabolism or exposure studies
- 3 Surveys of acrylamide in food
- 15 Studies relating to the epidemiology of acrylamide
- 4 Studies discussing the chemistry of acrylamide
- 6 Papers modelling acrylamide toxicity data

Reviews on acrylamide

- 32 Reviews or risk assessments on acrylamide

Others:

- 12 Papers relating to chemicals other than acrylamide
- 12 Papers exploring aspects of methodology
- 2 Articles in a foreign language
- 7 Articles published pre 1995
- 2 Items of PPG Correspondence

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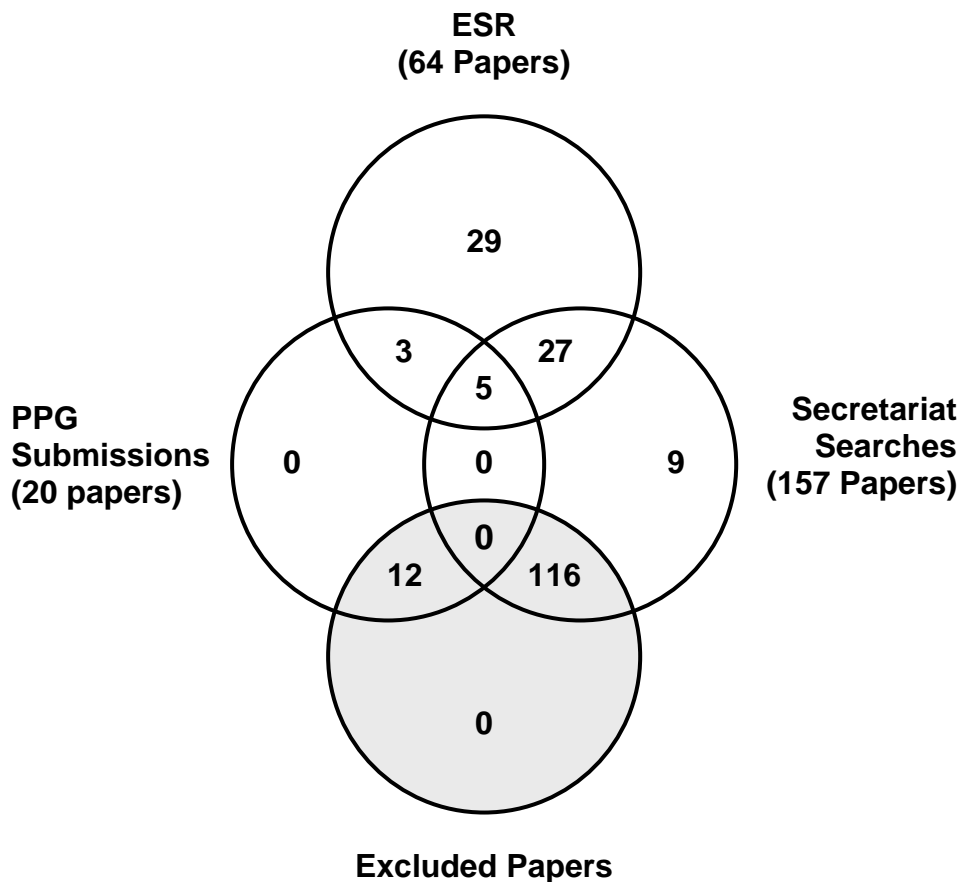
Secretariat Acrylamide Search Exclusions Pre 1995

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The following search was performed in Reference Manager™ (version 10) to consult the PubMed database†:

(acrylamide OR glycidamide) AND ("DNA damage" OR "DNA adduct" OR aneugen OR clastogen* OR genotoxic* OR mutagen*) AND 1966:1994 [pdat]*

The search identified 157 papers. The Venn Diagram below shows a comparison between the PPG Submissions, the EU Risk Assessment Report and the Secretariat Searches:



† <http://www.pubmed.gov>

The 125 papers identified by the Secretariat search, that were not also identified by the PPG submissions or the EU Risk Assessment Report, were sifted according to the following inclusion criteria:

- Abstract should indicate a primary report of mutagenicity data
- A title and abstract should be available in English

Of the 125 papers, 116 were excluded for the following reasons:

- 10 Toxicology Studies (2, Carcinogenicity; 1, Effects on GSH levels; 4, Toxicokinetics Studies; 1, Modelling of Toxicokinetic Data; 2, Neurotoxicity)
- 6 Review Articles
- 40 Used in Method (18, Polyacrylamide Gels; 18, Fluorescence Quenching; 1, Metabolic Induction in the production of S9 Liver fraction; 1, Biochemical use as a metabolic intermediate; 2, analytical techniques)
- 59 Wrong Chemical (Mostly 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide)
- 1 Mentioned Acrylamide in Key Words but not in the Abstract

The table below shows the remaining 9 studies which might be of relevance to the COM Review:

Study Type	Result	Reference
In Vitro Gene Mutation (Bacterial)	Positive Acrylamide	Ashby J;Tennant RW;Zeiger E;Stasiewicz S; (1989) Classification according to chemical structure, mutagenicity to Salmonella and level of carcinogenicity of a further 42 chemicals tested for carcinogenicity by the U.S. National Toxicology Program, <i>Mutat Res</i> 223 (2), 73-103
In Vitro Gene Mutation (Mammalian)	Positive Acrylamide	Dearfield KL;Harrington-Brock K;Doerr CL;Rabinowitz JR;Moore MM; (1991) Genotoxicity in mouse lymphoma cells of chemicals capable of Michael addition, <i>Mutagenesis</i> 6 (6), 519-525
In Vivo Chromosomal Aberrations	Positive Acrylamide	Kligerman AD;Atwater AL;Bryant MF;Erexson GL;Kwanyuen P;Dearfield KL; (1991) Cytogenetic studies of ethyl acrylate using C57BL/6 mice, <i>Mutagenesis</i> 6 (2), 137-141
In Vivo Chromosomal Aberrations	Positive Acrylamide	Fediukovich LV;Kotlovskii I;Sviderskaia LN;Borisov I; (1988) [Mutagenic and cytotoxic effects of acrylates], <i>Genetika</i> 24 (6), 1132-1134
In Vivo Gene Mutation (Drosophila)	Positive Acrylamide	Vogel EW;Nivard MJ; (1993) Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination, <i>Mutagenesis</i> 8 (1), 57-81
In Vivo Gene Mutation (Drosophila)	Positive Acrylamide	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, <i>Environ Mol Mutagen</i> 24 (2), 143-147
In Vivo DNA Adducts		Bailey E;Farmer PB;Shuker DE; (1987) Estimation of exposure to alkylating carcinogens by the GC-MS determination of adducts to hemoglobin and nucleic acid bases in urine, <i>Arch Toxicol</i> 60 (1-3), 187-191
In Vivo Germ Cell Dominant Lethal	Positive Acrylamide	Working PK;Bentley KS;Hurt ME;Mohr KL; (1987) Comparison of the dominant lethal effects of acrylonitrile and acrylamide in male Fischer 344 rats, <i>Mutagenesis</i> 2 (3), 215-220
In Vivo Germ Cell Dominant Lethal	Positive Acrylamide	Dobrzynska M;Lenarczyk M;Gajewski AK; (1990) Induction of dominant lethal mutations by combined X-ray-acrylamide treatment in male mice, <i>Mutat Res</i> 232 (2), 209-215

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Secretariat Acrylamide Search Exclusions

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Colleagues at the Information Centre searched other databases using the following search term:

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

Ingenta Connect¹

17 references identified. Of these 1 had not been identified by the Secretariat's PubMed search.

This reference met the inclusion criteria, seeming to report *in vivo* germ cell dominant lethal study. This will be considered for inclusion in the COM review:

Friedman MA; Adler I; Baumgartner A; Gonda H; Skerhut M (2000) 1-Aminobenzotriazole inhibits acrylamide-induced dominant lethal effects in spermatids of male mice, Mutagenesis 15 (2) 133-136

Barbour Index²

4 references identified, all JECFA / WHO reviews.

British Library OPAC³

88 references Identified. Of these 11 had not been identified by the Secretariat's PubMed search. This included one paper reporting an embryo micronucleus study, which had been identified in the PPG submissions.

¹ <http://www.ingentaconnect.com/>

² <http://www.barbour-index.co.uk/BarbourInfo/Our+Services/Professional+Services/Food+Safety.htm>

³ <http://direct.bl.uk/bld/Home.do>

The remaining 10 references were excluded: a review, a paper on poly acrylamide gels, a paper using acrylamide as a bacterial substrate, a food survey, a paper presenting non genotoxicity toxicology data and 5 references related a different chemical bearing an acrylamide moiety (mostly 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide).

FoodlineWeb⁴

37 references identified. Of these, 32 had not been identified by the Secretariat's PubMed search, but failed to meet the inclusion criteria. These included: 4 food surveys, 10 related to formation in food, 1 non genotoxicity toxicology study, and 17 reviews or risk assessments.

National Library for Health⁵:

Resources searched: Biomed Central (82), Dialog Datastar (0), NLH Evidence (0), NLH Guidance (0), NLH Specialist Libraries (0), Proquest (0)

Biomed Central references were considered relevant as they did not mention acrylamide or glycidamide in either the titles or abstracts. They were mostly cell/molecular biology papers.

Food Science and Technology Abstracts⁶

29 references identified. Of these, 8 had not been identified by the Secretariat's PubMed search. None met the inclusion criteria. These included: 5 reviews, 3 food surveys, and one paper on formation of acrylamide in food.

Current Contents⁷

133 references identified. Of these 35 had not been identified by the Secretariat's PubMed search.

3 references met the inclusion criteria; these seem to report studies on *in vitro* DNA damage, *in vivo* mouse germ cell mutation and *in vivo* drosophila germ cell mutation. These will be considered for inclusion in the COM review:

Galdo VC; 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,

⁴ <http://www.foodlineweb.co.uk/>

⁵ http://search.library.nhs.uk/nhs_sse/

⁶ <http://www.fstadirect.com/loginPage.asp>

⁷ <http://www.fstadirect.com/loginPage.asp>

induces DNA damage in rat thyroid cell lines and primary cultures, Mol. Cell. Endocrinol. 257-8, 6-19

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

Russell LB (2004) Effects of male germ-cell stage on the frequency, nature, and spectrum of induced specific-locus mutations in the mouse, Genetica 122 (1) 25-36

The remaining 32 were excluded for the following reasons: 1 food survey, 4 papers presenting non genotoxicity toxicology data, 2 toxicokinetic studies, a paper modelling adduct data, 4 reviews, 15 with methods using acrylamide (including electrophoresis and fluorescence quenching), 3 that referred to a different chemical bearing an acrylamide moiety, and 2 references where acrylamide was mentioned in the keywords but not the title or abstract.

Summary of Additional Searches

151 additional references have been identified by these searches.
4 of these meet the inclusion criteria and will be considered for the COM review.