Meeting

Discussion paper on EFSA’s 2022 Assessment of the genotoxicity of acrylamide

This is a paper for discussion.

This does not represent the views of the Committee and should not be cited.

Background

1. Following a request by the European Commission (EC), the European Food Safety Authority (EFSA) published a statement on the assessment of recent publications on the genotoxicity of acrylamide (AA) (EFSA, 2022). The request by the EC followed the publication of a review article by Eisenbrand (2020a) and its erratum (Eisenbrand, 2020b) that argued against a genotoxic mode of action for AA. However, as EFSA did not consider the review and erratum to be comprehensive, they undertook a literature search of the recent data on the genotoxicity and mode of action of AA.

2. FSA policy asked for the COT’s view on the recent EFSA (2022) statement. The paragraphs below briefly summarise the key points from the 2015 EFSA opinion on AA in food and the main considerations from the recent 2022 EFSA evaluation. The paper also provides a brief overview of the Eisenbrand publications.

3. Owing to the nature of the topic, the summary of the EFSA statement and Eisenbrand paper were initially presented to the Committee on Mutagenicity (COM) in June 2022. An outline of the COM’s conclusions is provided. FSA policy colleagues have however asked for a full risk assessment of acrylamide to be undertaken, which will be presented to the Committee in 2023.

4. An updated literature search on the genotoxicity of AA encompassing the time between EFSA’s search in 2021 and October 2022 has also been

conducted, and summaries of relevant papers identified are presented in this paper. Search terms and inclusion/exclusion criteria for the literature search are provided in Annex A. Please note, the COM were not provided with the Secretariats updated literature search during their discussions earlier in the year.

Summary of the EFSA assessments of the genotoxicity of acrylamide

Introduction

5. Acrylamide (AA) is a low molecular weight, highly water-soluble organic compound. Heightened concerns about exposure to AA arose in 2002 when it was discovered that it forms when certain foods are prepared at temperatures usually above 120 °C and in conditions of low moisture. It forms in part due to a Maillard reaction between certain amino acids and reducing sugars primarily in carbohydrate rich food, such as French fries, potato crisps, bread, and coffee.

6. The key toxicological effects of AA are genotoxicity, carcinogenicity, neurotoxicity, and effects on male reproductive parameters, including sperm counts and alterations in sperm and testis morphology.

7. AA is classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC, 1994). AA is a weak mutagen and effective clastogen in mammalian cells. Metabolic epoxidation of AA via CYP2E1 leads to the production of glycidamide (GA) which is a strong mutagen and clastogen. GA induces mutation via a DNA adduct mechanism and AA may induce DNA damage secondary to induction of oxidative stress.

8. Following the publication of a review article by Eisenbrand (2020a) and its erratum (Eisenbrand, 2020b), which concluded that the totality of available scientific evidence argues against a genotoxic mode of action underlying the neoplastic effects of acrylamide, EFSA published a statement on the assessment of recent publications on the genotoxicity of AA (EFSA, 2022).

2015 Evaluation by EFSA

9. EFSA (2015) evaluated the data on genotoxicity available at the time and concluded that;

- The in vitro studies indicate AA to be a weak mutagen in mammalian cells, yet an effective Clastogen.
- Glycidamide (GA), the epoxide metabolite of AA, was a strong mutagen and a clastogen, inducing mutations via a DNA adduct mechanism.
- In the in vivo studies, AA is clearly genotoxic in somatic and gem cells.
- The mechanism for AA's mutagenicity was via metabolism by CYP2E1 to GA.
- AA could also induce gene mutations by a pathway involving the generation of reactive oxygen species (ROS) and oxidative DNA damage.

10. EFSA also noted that in situations of limited CYP2E1 activity, genotoxicity of AA may involve the generation of ROS and oxidative DNA damage. However, this alternative pathway appears to only take place at very high doses, and it has been suggested that the clastogenic effect may be mediated through interference with the kinesin motor proteins involved in spindle fibre formation and chromosomal segregation during cell division or alkylation of protamine in sperm. An alternative suggestion is the alkylation of proteins associated with chromatin via AA's affinity for sulfhydryl groups.

11. As the number of tumours originating from tissues involved in the endocrine system were significantly increased in the rat bioassays, AA was hypothesised to act as a carcinogen via adverse effects on endocrine regulation. However, EFSA noted that the studies investigating whether local endocrine effects explained tumour formation in certain hormone or paracrine-regulated target tissues reported inconsistent changes in thyroid hormones and hence concluded that the mechanistic hypothesis lacked experimental proof.

12. EFSA concluded that the epidemiological studies did not provide consistent indication for an association between AA exposure and increased cancer risk in various organs; the studies available had severe limitations, uncertainties in the assessment of exposure and lacked statistical power.

13. Due to the concern in respect to genotoxicity, EFSA did not consider it appropriate to establish a health-based guidance value (HBGV) and instead applied the margin of exposure approach for compounds that are both genotoxic and carcinogenic. EFSA thereby used the BMDL10 of 0.17 mg/kg bw per day, the lowest BMDL10 from a 2-year study in male mice, showing an increased incidence of Harderian gland adenomas and adenocarcinomas.

14. Harderian glands are absent in humans, however EFSA noted that it is a sensitive target tissue in rodents for compounds that are both genotoxic and carcinogenic. Taking into account that target tissues for tumour formation can

vary between species for a given compound, EFSA considered the Harderian gland a conservative endpoint for the assessment of neoplastic effects of AA in humans.

2022 Evaluation by EFSA

Genotoxicity

15. EFSA assessed all studies on genotoxicity published since their last opinion in 2015; Brief summaries of the findings are provided below; a more detailed description of the studies can be found in Table 1 (in vitro) and Table 2 (in vivo) of the EFSA opinion.

Chromosomal damage

In vitro

16. Micronucleus tests following in vitro exposure of human or rat lymphocytes were positive, the emphasis of these studies was on the ability of L-carnitine and resveratrol to prevent AA-induced DNA damage (Zamani et al., 2018; Ankaiah et al., 2018). In a study by Liu et al. (2015) AA was found to increase the percentage of chromosome misalignment in Metaphase II-stage mouse oocytes in female rats in a dose-dependent matter. AA further induced significant alterations in spindle morphology. The results were found to be associated with a decrease in maturation of mouse oocytes. The results in all studies were independent of added metabolic activation systems.

In vivo

17. In mice, in vivo oral exposure was consistently positive in the micronucleus test in the bone marrow or peripheral blood (Hobbs et al., 2016; Algarni et al., 2018; Hagio et al., 2021). A more limited set of studies by Zhao et al. (2015 a;b) resulted in AA induced alterations of the liver antioxidant enzymes and micronuclei and DNA breaks after a single i.p. injection. The changes were alleviated by feeding mice a diet containing various fruits or extracts containing antioxidants. Chromosome aberrations in mouse bone marrow cells showed increased levels of polyploidy and chromosome fragments, deleted chromosomes and Robertsonian centric fusions (Algarni, 2018); maturation of mouse oocytes was significantly impaired by AA treatment, with metaphase II oocytes showing a reduction in meiotic spindle mass and chromosome misalignments (Aras et al.,

2017).

18. Wister and Sprague-Dawley rats treated orally with AA showed increased micronuclei (Jangir et al., 2016; Shimamura et al., 2017; Sekeroglu et al., 2017), while Fisher 344 rats did not (Dobrovolsky et al., 2016; Hobbs et al., 2016; Chepelev et al., 2017).

19. EFSA concluded that mice seemed to be more sensitive to micronuclei formation than rats, which correlates with the level of DNA adducts being generally lower in rats than mice after AA exposure.

Comet assay

In vitro

20. DNA strands breaks were shown in human THP1-monocytes, liver HepaRG cells and CaCo2 cells, lymphocytes and spermatozoa following in vitro exposure to AA. (Xiao et al., 2016; Mandon et al., 2019; Nowak et al., 2020; Wang et al., 2021; Katen et al., 2017). An increase in DNA strand breaks in mouse spermatozoa was only observed following exposure to GA, or with AA in the presence of conditioned media from epididymal mECap18 cells that expressed high levels of CYP2E1 enzyme required for metabolic activation of AA (Katen et al., 2017).

21. In the presence of a DNA glycosylase (Fpg, which removes 8-oxoguanine and other DNA lesions) substantial increases in DNA strand breaks were observed in CaCo2 cells, spermatozoa and human lymphocytes after AA exposure (Katen etal., 2017; Hansen et al., 2018; Nowak et al., 2020). In assays supplemented with the human OGG1 enzyme (8-oxoguanine DNA glycosylase involved in maintaining genomic integrity) only modest changes in levels of DNA breaks were observe. The findings indicate that the majority of AA or GA induced DNA breaks are not due to the presence of DNA 8-oxoguanine but that at least a fraction of the induced DNA breaks might be due to DNA oxidation (Katen et al., 2017; Hansen et al., 2018). Elevation of ROS levels, together with mitochondrial depolarisation have been observed in parallel with DNA strand breaks in CaCo2 cells (Nowak et al., 2020) and modulation of these by antioxidants in human lymphocytes in vitro (Wang et al., 2021).

22. Increased levels of DNA strand breaks and sister chromatid exchanges were observed in lymphocytes from individuals carrying polymorphism in the

CASP10 and CASP8 genes, respectively, after GA exposure. The authors suggested that these caspase polymorphisms may decrease the apoptotic rate, increase cell survival and consequently yields of genotoxic effects of GA (de Lima at al., 2016).

In vivo

23. In vivo, lymphocytes and liver of mice (i.p.), liver (i.p.), kidney and brain (oral) in Wistar rats and liver in F344 rats (oral) were positive after AA exposure (Zhao etal., 2015a;b; Ansar et al., 2016; Dobrowolsky et al., 2016; Shamimamura et al., 2017). Spermatocytes and spermatozoa of AA exposed mice (i.p. or a 6 month oral treatment) showed increased levels of DNA damage. Male offspring of male mice treated with AA also showed increased levels of DNA breaks in spermatozoa (Katen et al., 2016a;b; 2017).

24. EFSA conclude that the data indicated AA induced DNA damage in several rodent organs. In addition, chronic paternal exposure in rats had consequences for male offspring.

Gene mutation

In vitro

25. AA and GA increased the TK gene mutation frequency in the human MCL-5 lymphoblastoid cell line (David and Gooderham, 2018). At the highest tested AA dose, Hupki mouse embryo fibroblasts showed a 2-fold increase in mutation frequency of the knock-in LacZ gene while a 1.5-fold increase was observed in the metabolically competent FE lung cells (Hoelzl-Armstrong et al., 2020a;b). In the presence of S9, gpt gene mutations were induced in lung cells in pulmonary organoid structures of mice (Komiya et al., 2021), the mutational spectrum was similar to that reported previously in vivo in transgenic mice (Ishi et al., 2015).

26. Data from various cell lines indicate that GA is more potent than AA. Especially at concentrations that caused similar levels of cytotoxicity, GA induced three times more lacZ mutations than AA (Hoelzl-Armstrong et al., 2020a).

27. In contrast, in Hupki mouse embryo fibroblasts the analysis of a human knock-in TP53 gene nor exome or whole genome sequencing showed evidence of AA-induced mutations, which the authors attributed to the limited ability of Hupki cells to activate AA (Hoelzl-Armstrong et al., 2020a; Zhivagui et al., 2019). Exposure to GA in the absence of metabolic activation, however, did increase

mutation frequency at the TP53 gene, the majority of the mutations occurred at the A:T base pair and at specific TP53 codons that have also been found to be mutated in human tumours (Hoelzl-Armstrong et al., 2020a).

28. Two further in vitro studies in Hupki mouse embryo fibroblasts, analysed mutational spectra induced by GA and found a 2.5-fold increase in the number of single base substitutions (SBS) at 3 mM and a non-statistically significant increase in mutational load at 1.1 mM. Comparison of the specific GA signature/mutational spectrum to the Pan-Cancer Analysis of Whole Genomes (PCAWG) database SB mutational signatures and the Catalogue of Somatic Mutations in Cancer (COSMIC) database led the authors to conclude that AA and/or GA associated mutagenesis contribute to human cancers (Zhivagui et al., 2019; Hoelzl-Armstrong et al., 2020a).

In vivo

29. In vivo studies investigating the mutagenic potential, mainly in reticulocytes and red blood cells (at the endogenous Pig-a gene) were either equivocal (no response, positive at a single dose or single cell type) or negative for rats and mice treated with AA (Dobrovolsky et al., 2016; Hobbs et al., 2016; Horibata et al., 2016; Chepelev et al., 2017).

30. Increased cII gene mutation frequency was observed in the brain of mice exposed to AA and GA and reported at the gpt gene in the testis, lung (2-3-fold) and sperm (6-fold) of mice treated with AA and mutagenicity in male rats indicated that cells in the late stage of spermatogenesis are more sensitive than spermatogonial cells. The mutational classes varied between organs. (Li et al., 2016; Hagio et al., 2021).

31. EFSA concluded that both in vitro and in vivo data highlighted the relationship between DNA adduct profiles originating from the metabolic conversion of AA and the mutational signature of AA/GA.

DNA adducts

32. A brief summary of the main observations on DNA adduct formation is provided below, a list of the individual studies can be found in Table 3 of the EFSA opinion.

In vitro

33. AA induced DNA adducts (N7-GA-Gua and N3-GA-Ade) in calf thymus DNA in vitro and in the presence of S9 in Hupki mouse embryo fibroblasts, no DNA adducts were seen in the absence of S9. In contrast, high levels of DNA adducts were seen in the same cells following GA exposure (Hansen, et al., 2018; Zhivagui et al., 2019). Another study in Hupki cells also reported DNA adduct formation after GA but not AA exposure. In primary cultures of rat hepatocytes exposed to AA, a non-linear concentration response was observed, the increase in DNA adduction over background levels was only seen at concentrations of 1000 and 2000 µM (Hemgesberg et al., 2021a).

In vivo

34. In smokers, non-smokers and all study subjects combined, a significant correlation (but not-statistically significant difference between values) was seen between urinary N-acetyl-S-(propionamide)-cysteine (AAMA) mercapturic acid derivative (a metabolite of AA and marker of current exposure) and N7-GA-Gua levels, but not urinary cotinine and other factors. The authors therefore concluded that urinary N7-GA-Gua is significantly associated with dietary AA intake (Huang et al., 2015). Significantly higher levels of urinary N7-GA-Gua, AAMA and the mercapturic acids of AA and GA (namely N-acetyl-S(2-carbamoylethyl)-L-cysteine (AAMA) and N-(R,S)-(1-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA)) were found in AA exposed workers (N=8) than in the control group (N=36) (Huang et al., 2018).

35. Low levels of N7-GA-Gua were detected in 80 % of healthy human volunteers (N=56), however no correlation between DNA adducts and dietary habits were found. Blood glucose levels or glycated haemoglobin and DNA adducts did significantly correlate with the body mass index (Hemgesberg et al., 2021b). Following a 24-hour dietary exposure of healthy volunteers (N=17) to AA present in carbohydrate rich foods as part of a normal human diet, no direct correlation could be seen between the AA intake, based on the food frequency questionnaire, and N7-GA-Gua levels. The authors did however stress that this might be due to the sample size and possible inaccuracies of the questionnaire (Jones et al., 2021). It was noted (presumably by EFSA) that the levels of DNA adduct formation were in the same range in these two human volunteer studies.

36. A dose dependent increase in N7-GA-Gua and N3-GA-Ade levels was reported in the liver and lung of mice orally treated with AA for 28 days at concentrations ranging from 87.5-700 µM (De Conti et al., 2019). EFSA found the

data to provide evidence on a dose linearity in the adduct formation in the dose range applied. N7-GA-Gua adducts were also reported in urine and tissues of rats exposed to AA for 7 and 14 days (Wang et al., 2019).

Non-genotoxic effects and genotoxicity secondary to oxidative stress

37. The following provides a brief summary of the new studies identified on non-genotoxic effects of AA and genotoxicity secondary to oxidative stress that may contribute to AA carcinogenicity. Summaries of the studies identified can be found in Table 4 of the EFSA opinion.

38. Several studies (in vitro and in vivo) provided further evidence for oxidative stress in various cellular systems and tissues in vivo and for associated oxidative damage to DNA. While a number of studies reported DNA breaks due to enzymes involved in the repair of oxidative damage (Fpg, EndoIII, OGG1), the interpretation of these findings should be taken with caution, according to EFSA. Hansen et al., (2018) provided evidence that DNA damage revealed through the use of Fpg can also be directly induced by GA and since Fpg was more effective in inducing DNA breaks during repair than OGG1 oxidative DNA damage may be a relatively small component of the damage detected in the Comet assay (Katen et al., 2017). Several studies did however show the formation of 8-oxoguanine via ROS production and demonstrated an association between micronuclei formation and oxidative stress (Wang et al., 2015; Katen et al., 2016a;b; Sekeroglu et al., 2017; Zamani et al., 2018; Salimi et al., 2021a;b; Zhao et al., 2015a;b; Ankaiah et al., 2018).

39. Oxidative damage has been reported to be reduced by antioxidants (Zhao et al., 2015a;b; Ansar et al., 2016; Nowak et al., 202; Wang et al., 2021), with one study showing inhibition of CYP2E1 and hence inhibition of the metabolic oxidation of AA to GA. However, EFSA noted that the studies did not necessarily report inhibition of DNA damage via antioxidant activity.

40. Transcriptomic changes in the thyroid of Wistar rats exposed to AA were reported by Colli-Dula et al. (2016), including genes involved in DNA damage and repair and other changes related to e.g. oxidative stress and motor proteins and kinases. In contrast, Chepelev et al (2017) did not find evidence of a DNA damage response in F344 rat thyroid but it was observed in the liver, including the induction of p53. A third study in rats (F344) did not show elevated expression of the p53 gene in testis in vivo (Recio et al., 2017). EFSA noted that the strains of rats used in these three studies differed. In mice, genes involved in the p53

pathway were not significantly enriched in the Harderian gland or the lung, but an elevation of phosho-p53, phosho-Chk1 and g-H2ax proteins suggested a DNA damage response in mouse embryo fibroblasts treated with AA or GA (Chepelev et al., 2018; Hoelzl-Armstrong et al., 2020a).

41. Changes in the expression of genes involved in calcium signalling and the cytoskeleton have also been implicated as a major transcriptome response. It has been suggested that the changes in calcium signalling and actin filaments may lead to impaired microtubule and microfilament integrity and hence interfere with chromosome segregation during cell division (Chepelev et al., 2017; 2018; Recio et al., 2017). These effects may contribute to the observed misaligned chromosomes and abnormal spindle morphology seen in AA exposed oocytes (Liu et al., 2015). However, EFSA noted that the specific role of calcium signalling is, to date, unproven.

42. A study by Ekanem et al. (2019) produced evidence of enhanced cell proliferation by an increased expression of cell cycle regulators and in the migratory ability of prostate cancer cells after GA exposure.

43. In utero exposure of rats to AA resulted in transcriptomic changes involved in genes related to cell cycle, specifically plasma T3 and T4 were increased. This further supported EFSA's previous conclusion that the data suggesting disturbance of thyroid hormones by AA was inconsistent. While EFSA recognised that modulation of thyroid hormone levels has been associated with thyroid carcinogenesis in rodents, the mechanism of the thyroid dysregulation is not known and there is no clear evidence that AA results in raised TSG. EFSA also noted that the relevance to humans is questionable (Bartsch et al., 2018).

Other relevant data

Endogenous formation of AA

44. Based on the available evidence, EFSA was previously unable to draw a conclusion on the possible endogenous formation of AA and considered the possibility that cysteine adducts of AA in dietary proteins could be present in food and feed, which could be absorbed from the gastrointestinal (GI) tract and subsequently excreted in urine.

45. Since the last EFSA evaluation of AA in 2015, two further studies have been published, in healthy volunteers (non-smokers) under strictly controlled conditions. In the study by Ruenz et al. (2016) volunteers were exposed to low

and high dietary levels of AA, following a 3-day wash-out period with an AA-minimised diet. Based on a 58 % excretion of AA in urine (as AAMA) within three days and the urinary AAMA concentration at the end of the wash out period the authors estimated an endogenous AA formation of 0.2-0.3 ug/kg bw per day. The authors themselves, however, noted the possibility of a high dietary AA exposure prior to the wash out period, resulting in residual AAMA excretion. The follow up study performed (Goempel et al., 2017) included a group who received a minimized AA diet for the whole duration of the study period (13 days) and were given 13C3D3-AA on day six. A second group received the minimized diet and a low AA exposure on day 6 and a high AA exposure on day 10. While the urinary excretion of AAMA was in the range of the control group after exposure on day 6, an approximately 10-fold increase of AMMA but also GAMA was seen after high exposure on day 10. The high AA exposure resulted in statistically significant increases in N-terminal valine haemoglobin adducts (of AA and GA). The authors estimated the endogenous AA formation to be 0.3-0.4 ug/kg bw per day and suggested Maillard type chemistry.

46. A further potential precursor for endogenous AA formation is acrolein, with oxidative stress being suggested as an endogenous source (Tareke et al., 2008; Ruenz et al., 2019; Cleusix et al., 2008; Zhang et al., 2018). Lipid peroxidation and acrolein formation have also been suggested as the basis for the association between GA-N7-Gua in peripheral blood mononuclear cell (human volunteers) with the body mass index (BMI) (Hemgesberg et al., 2021b).

47. EFSA concluded that endogenous formation of AA (in the range of 0.2-0.4 ug/kg bw per day) adds to the dietary exposure (mean 0.4-1.9 and P95 0.6-3.4 ug/kg bw per day).

Measurement of haemoglobin adducts

48. EFSA considered it beyond the scope of the current assessment to review all available studies/data on Hb adducts of AA and GA in humans exposed to background levels of AA in their diet, due to the large size of the database. However, EFSA previously discussed the results from the 2003-2004 US National Health and Nutrition Examination Survey (NHANES) in their 2015 opinion. Subsequent surveys continued to measure Hb adducts of GA in the US population. In Europe, the European Prospective Investigation into Cancer and Nutrition (EPIC) measured Hb adduct formation of both AA and GA.

49. EFSA concluded that the data demonstrates that GA is produced as a result of dietary exposure to AA and is not entirely detoxified. It is therefore systemically available.

EFSA discussion and conclusion

50. EFSA concluded that the majority of the new studies published since 2015 confirmed and extended the clastogenic properties of AA/GA. Analysis of chromosomal aberrations in mouse bone marrow showed increased polyploidy and chromosome breakage. EFSA suggested that the relatively low metabolic activating capability of F344 rats may explain the opposing findings in some studies.

51. New studies reporting DNA damage in several organs of mice and rats and in lymphocytes, spermatocytes and spermatozoa were in agreement with EFSA’s previous conclusions that increased DNA damage is associated with AA exposure, including effects on the male reproductive system.

52. Recent in vitro and in vivo data are in line with EFSA’s conclusions from 2015 and highlight the relationship between DNA adduct profiles originating from the metabolic conversion of AA to GA and the mutational signature of AA/GA. The studies also may indicate a contribution of AA and/or GA-associated mutagenesis to human cancers. The recent identification of an AA mutational fingerprint highlights the specificity of the mutational events associated with AA exposure.

53. More recent in vivo and in vitro studies confirmed the presence of the previously identified N3-GA-Ade and N7-GA-Gua adduct formation. However, EFSA noted the large variations in the number of adducts among the in vitro studies induced by AA and GA. The presence of DNA adducts (N7-GA-Gua) has also been reported in the urine of AA exposed workers and human volunteers. However, in two studies no association was found between DNA adduct formation and specific dietary habits. However, EFSA/the authors concluded that the lack of an association may be due to the relatively small sample size and limitations in the exposure assessment.

54. EFSA concluded that endogenous formation of AA has been demonstrated at levels below dietary AA exposure and hence the impact of the addition of dietary exposure to endogenous formation needs to be considered. EFSA also concluded/confirmed that GA is not entirely detoxified on formation and hence it is systemically available in humans with common levels of dietary AA exposure, meaning dietary exposure to AA has the potential to result in the

formation of GA adducts and GA-related mutations.

55. In addition to genotoxicity EFSA also considered the potential for both secondary DNA oxidation (via ROS) and effects on the control of the cell cycle that may contribute to carcinogenesis. The origin of the ROS was not considered clear while changes in histone acetylation and methylation and DNA methylation in liver and lung of mice showed increased expression of cell cycle regulators, which in turn point to epigenetic influences that may contribute to enhanced cell proliferation and target organ carcinogenesis. Although altered calcium signalling may involve modulation of microtubules and microfilaments and the action of kinesis during cell division, EFSA did not consider these effects to contribute to carcinogenicity. EFSA did consider they may be important in neurotoxicity, however.

56. Overall, EFSA concluded that in addition to genotoxicity, non-genotoxic effects may contribute to the carcinogenicity of AA. There is further substantial evidence for the genotoxicity of AA to be mediated by the formation of GA.

57. The new studies evaluated by EFSA extend the information assessed previously and support its conclusion on the risks to human health related to the presence of AA in food.

58. EFSA further considered the MOE approach to still be appropriate and concluded that an update of its 2015 opinion is currently not required.

Publication by Eisenbrand (2020)

59. The recent review article by Eisenbrand (2020) which prompted the EFSA review concluded that the totality of available scientific evidence clearly argues against a genotoxic mode of action underlying the neoplastic effects of acrylamide.

60. The article further suggested that endogenous formation of AA occurs at a rate close to average AA exposure from the diet and that GA is entirely detoxified at dietary exposure levels by conjunction with glutathione.

61. A brief summary of the main points taken from the abstract of the review article has been provided below. The information provided and conclusions drawn are the authors', not the Secretariats'.

62. The author argues that in primary rat hepatocytes, the biotransformation of AA into GA is substantially slower than the detoxifying

coupling to glutathione (GS).

63. According to the author high dose ranges (> 100 µg/kg bw) were required for DNA adduct (N7-GA-Gua) formation in rats, while lesions were only detected periodically at levels of average consumer exposure, without dose dependence. Furthermore, substantial built up of AA-haemoglobin (Hb) adducts were reported in rats, while GA-Hb adducts remained within background levels.

64. The author further argues that the effects on calcium signalling and cytoskeletal functions in rodent target organs from toxicogenomic studies, and strain- and species-specific neoplasms from carcinogenicity studies in rodents are not likely to be predictive of human cancer risk.

65. The author considers GA a weak mutagen, supported by comparison to other process-related contaminants, such as N-nitroso compounds, polycyclic aromatic hydrocarbons or potent food borne mutagens/carcinogens.

66. The author concludes that a non-genotoxic/non-mutagenic MOA is underlying the neoplastic effects of AA in rodents and hence a tolerable intake level (TDI) may be defined. Thereby the key adverse effects should be considered, supported by biomarker-based dosimetry in experimental systems and humans.

Summary of the discussion and conclusion by the COM

67. The COM agreed that the information/data EFSA considered in their assessment confirmed and strengthened most aspects of the previous opinion.

68. However, Members noted that while there was sufficient evidence that GA was mutagenic, the direct evidence for AA was less clear, but predominantly relied on its metabolism to GA. Members further noted that uncertainties raised in the previous opinion still continued to lack sufficient data to close said gaps. Members would like to see studies in relevant species, at levels that would allow for the identification of benchmark dose effects. There further remained uncertainty about endogenous levels.

69. Members noted that the Eisenbrand paper lacked detail in the approach to choosing/reviewing its selected studies and it appeared to consider specific evidence (e.g., focused on one specific DNA adduct), rather than considering all evidence. Overall, the COM agreed with EFSAs conclusion that the MOE approach

would still be appropriate, and an update of the 2015 opinion is currently not required.

Updated literature search following EFSA's 2022 assessment of the genotoxicity of acrylamide

70. A literature search on the genotoxicity of AA was performed covering the time between EFSA's search in 2021 and mid-October 2022. The following paragraphs provide summaries of the publications retrieved including in vitro cellular genotoxicity, in vivo experimental toxicity studies, and epidemiological studies, as well as several studies utilising acrylamide as a positive control in assay development for detection and quantification of genotoxicity (both in vitro and in vivo).

71. A number of studies reporting on the relationship between acrylamide exposure and other non-genotoxic/non-carcinogenic adverse outcomes were also retrieved, including effects on metabolism, immune function, and reproductive and developmental toxicity. For completeness, the key conclusions from these studies have been summarised here. However, it should be noted, that a comprehensive search for non-genotoxic/carcinogenic endpoints was not performed and hence this list is not comprehensive.

72. The literature search was performed after the COM's review of the EFSA statement and Eisenbrand publications in June 2022, and have therefore not been reviewed by the COM.

73. The literature search strategy is detailed in Annex A.

Genotoxicity/carcinogenesis

In vitro studies

74. A study by Gouveia-Fernandes et al. (2022) investigated the effect of the acrylamide bioactivation metabolite, glycidamide (GA), on hepatocarcinogenesis in the non-malignant THLE2 and malignant HepG2 liver cell lines. Glycidamide treatment induced a range of molecular alterations in liver cells which the authors suggested were consistent with transition to a pro-carcinogenic phenotype.

75. Both cell types were treated with glycidamide at 0.1, 1, 5, 7.5, 10, 15, 20 and 30 mM for 24 hours. Glycidamide treatment dose-dependently induced cell death via apoptotic, necrotic, and necroptotic pathways with an LC50 of 8.8 mM and 15.4 mM in THLE2 and HepG2 cells, respectively. Cell cycle analysis demonstrated that the HepG2 cells surviving glycidamide-induced toxicity had an increased proportion of cells in G2/M phase at 20 and 30 mM glycidamide which the authors suggested indicated an enhanced proliferation rate.

76. The authors analysed mRNA levels of four different enzymes (glutamate cysteine ligase catalytic subunit (GCL-C), catalase (CAT), and the glutathione S-transferases GSTP1 and GSTA3) involved in antioxidant defence. Results showed that surviving cells had altered expression. In non-malignant THLE2 cells, GCL-3 was unaffected, GTSP-1 and CAT were downregulated by high concentrations of glycidamide (15.4 mM for GTSP-1; 5, 8.8 and 15.4 mM for CAT), and GTSA3 was upregulated with glycidamide treatment (8.8 and 15.4 mM). The authors concluded that “the downregulation of GSTP1 and the upregulation of GSTA3 by high concentrations of glycidamide…can indicate…a putative malignant transformation of THLE2 cells.” In HepG2 cells, GCL-C was upregulated at low levels of glycidamide and reduced at higher levels. GSTP-1 was undetected, GSTA3 levels were higher than in THEL2 cells and exhibited non-dose dependent changes in expression (reduced at 5 and 8.8 mM, unaffected at other doses), and CAT was downregulated at high concentrations (5, 8.8, and 15.4 mM glycidamide). The authors suggested that basal expression levels of these enzymes in HepG2 cells reflected their cancerous phenotype, and changes upon glycidamide treatment might have represented adaptive (low doses) and deleterious (high doses) responses to oxidative stress.

77. The authors further suggested that changes in enzyme expression levels may have been mediated through HDAC genes, levels of which were altered with glycidamide treatment. HDAC2 and HDAC9 were decreased at high concentrations in THEL2 cells (8.8 and 15.4 mM, and 15.4 mM, respectively) whilst HDAC2 and HDAC8 were increased at lower (1 and 5mM, and 1 mM, respectively) and increased at higher concentrations (8.8. and 15.4 mM for both HDACs) in HepG2 cells.

78. Expression of the liver cancer phenotype marker alpha-fetoprotein gene (AFP) was increased in THLE2 cells with glycidamide treatment at 8.8 and 15.4 mM, and decreased in HepG2 cells, although the latter cells had higher basal expression levels. Expression of tumour suppressor gene TP53 was unaffected in THEL2 cells, whilst it was increased at low doses (1 and 5 mM) and decreased at

higher doses (8.8 and 15.4 mM) in HepG2 cells. The authors concluded that changes in AFP expression in THEL2 cells, and TFP53 expression in HepG2 cells, suggest malignant transformation and response to DNA damage, respectively. Glycidamide treatment also increased the proportion of cells expressing the stemness marker CD133 (7.5, 10, and 15 mM for THEL2 and 10, 15, and 20 mM for HepG2), which, according to the authors, further corroborated the carcinogenic role of glycidamide.

79. Kontaş Yedier et al. (2022) studied the effects of acrylamide on cytotoxicity, genotoxicity, and carcinogenesis in the lung epithelial cell line BEAS-2B. The authors concluded that acrylamide “exposure can induce carcinogenesis in lung cells and may be a risk for lung cancer formation.”

80. Cells treated with acrylamide at 0.005, 0.01, 0.05, 0.1, 0.5 1, 5, and 10 mM underwent dose-dependent cell death with LC50 values of 6.6, 1.97, and 1.33 mM at 24, 48, and 72 hours, respectively. In the Comet assay, acrylamide treatment for 24, 48, and 72 hours at 0.5, 1, and 2 mM increased percentage of DNA in the tail, tail moment, and olive tail moment. Increases were significant and non-concentration dependent. The authors concluded that the comet assay results indicated that acrylamide “could induce the formation of DNA single strand breaks.”

81. The authors further analysed expression of gH2AX and 53BP1 in BEAS-2B cells after treatment with acrylamide, which act as markers for DNA damage and repair (Kuo and Yang, 2008) and double-strand break repair (Panier and Boulton, 2014), respectively. Acrylamide treatment at 0.5, 1 and 2 mM significantly increased the formation of single- and double-positive gH2AX and 53BP1 foci in the nuclei of the cells in a concentration-dependent manner. The authors suggested this indicated the induction of double strand breaks.

82. Following acrylamide treatment for 72 hours (0.5, 1 and 2 mM) BEAS-2B cells underwent morphological changes, including the appearance of long-spindle structures and vacuoles. Cells also transitioned from monolayer growth to multi-layered and invasive foci that exhibited more rapid proliferation. After seeding in soft agar, acrylamide treated cells exhibited dose-dependent increases in anchorage-independent growth and formed larger colonies than untreated cells. The authors concluded that “these changes are very similar to the characteristics of malignant cells.”

In vivo studies

83. Hashem et al. (2022) investigated effects of low-dose acrylamide on haematological parameters, immune indicators, splenic and bone marrow tissue architecture, and CD4+ and CD8+ immunoexpression in male Wistar rats. As part of this study, they also studied the effects of AA on the integrity of the DNA of splenic cells. Male Wistar rats were exposed to 1 or 2 mg/kg bw/day acrylamide for 90 days and spleen cell DNA integrity was analysed by comet assay. AA exposure dose-dependently increased tail length, percentage of DNA in the tail, and tail moment. The authors suggested that "this DNA damage could have been caused by interactions between ROS and DNA that occurred due to AA exposure" in their model, and that their findings "corroborated AA's potential for genotoxicity that had been demonstrated before in animal models and cells."

84. Acrylamide also dose-dependently induced leukopenia, lymphocytopenia, eosinophilia, and thrombocytopenia in Male Wisatr rats. IgG and IgM levels were also increased, whilst makers of innate immunity (phagocytic activity, lysozyme, and nitric oxide) were reduced. Tissue degeneration was observed in the spleen and bone marrow, and the spleen showed increases in CD4+ and CD8+ positive cells.

Epidemiological studies

85. Bellicha et al. (2022) conducted an epidemiological study to investigate the association between dietary acrylamide exposure and breast cancer risk. This was a prospective study of the NutriNet-Santé cohort of 80,597 French females (age 40.8 ± 14 years) who were followed up for 8.8 ± 2.3 years.

86. Using dietary records referenced against acrylamide measurements from the database of the Second French Total Diet Study, mean acrylamide intake was estimated at 0.49 ± 0.36 µg/kg bw/day. The main contributors to acrylamide exposure were coffee, potato crisps and chips, pastries and cakes, and bread. Acrylamide exposure levels were ranked by quartiles, and average exposures in Q1-4 (µg/kg bw/day) were: 0.15 ± 0.06, 0.32 ± 0.08, 0.53 ± 0.11, and 0.96 ± 0.36. During follow-up of 8 ± 2.3 years (followed up every 6 months), 1016 first incident breast cancer cases were diagnosed, which consisted of 431 premenopausal cases and 585 postmenopausal cases.

87. In analysis of the whole cohort, a non-linear association was observed between levels of daily acrylamide consumption and breast cancer risk with higher risk observed in the second and fourth quartiles of exposure. The overall association was borderline significant ($p=0.05$), and further sub-analysis demonstrated that acrylamide exposure was significantly associated with

premenopausal, but not postmenopausal breast cancer incidence. Breast cancer risk in premenopausal women followed the same non-linear acrylamide dose-response pattern observed in the whole cohort analysis (higher in quartiles two and four, with no effect in the second quartile). Hazard ratios for breast cancer risk in premenopausal were 1.36 (Q2), 1.09 (Q3), and 1.40 (Q4), with Q1 being the reference group. In this sub-analysis, participants contributed up to their age at menopause at the latest for the premenopausal model, and from their age at menopause to the postmenopausal model.

88. Further sub-analysis suggested a positive association between acrylamide exposure and oestrogen-receptor positive breast cancers, although the authors commented that the statistical power of this sub-analysis was limited by the low occurrence of hormone receptor-negative breast cancers in the cohort.

89. Overall, the authors concluded that this study demonstrated an association between acrylamide exposure and breast cancer occurrence in premenopausal women. The effect was non-linear, with lower and higher, but not intermediate exposures showing association with breast cancer. Through reference to the observation of non-linear effects of acrylamide on carcinogenesis in the wider literature, the authors suggested this might be linked to acrylamide escaping detoxification mechanisms at low levels and overwhelming them at higher levels

90. EFSA’s initial Scientific Opinion (2015) on acrylamide assessed results from the US National Health and Nutrition Examination Survey (NHANES) cohort relating to dietary acrylamide exposure and levels of acrylamide biomarkers in the blood (acrylamide- and glycidamide-haemoglobin (Hb) adducts; AA-Hb and GA-Hb). Since EFSA’s 2022 assessment of the genotoxicity of acrylamide a new follow-up study investigating this cohort has been published (Gu et al., 2022).

91. Gu et al. (2022) aimed to investigate the association between levels of acrylamide biomarkers at the time of measurement and mortality from all forms of cancer in an average follow up period of 10.3 years. Levels of AA-Hb and GA-Hb were measured once between 2003 and 2005 using high-performance liquid chromatography/tandem quadrupole mass spectrometry. Mortality was ascertained by the National Death Index (NDI) records up to the end of 2015. Cause-specific death was determined using International Classification of Diseases, Tenth Revision (ICD-10) and cancer mortality was defined as ICD-10 codes.

92. Out of 3717 total subjects, 118 died of cancer (all forms) during the follow up period of 10.3 years. The authors used four separate cox regression models to investigate the association of AA-Hb and GA-Hb with cancer mortality. These models sequentially adjusted for different co-variates: model 1 – age, sex, race; model 2 – smoking and drinking status, education level, income, BMI, exercise, energy consumption, and cotinine; model 3 – diabetes, hypertension, and dyslipidaemia; model 4 – low-grade inflammation score. Haemoglobin acrylamide adduct levels were ordered by quintiles and hazard ratios of >1 were observed in the highest quintile for AA-Hb levels (in all four models), in the quintile 3-5 for GA-Hb levels, and quintile 4-5 for AA-Hb + GA-Hb (in all four models). The Ptrend indicated a significant linear correlation between AA-Hb levels and cancer mortality in model 1 only, and in all four models for GA-Hb and AA-Hb + GA-Hb levels. A logistic regression analysis of cancer sub-types demonstrated an association between acrylamide exposure and uterine cancer (HR 4.26, 95% CI 1.04-17.54).

93. Spline analysis (adjusted for all covariates) demonstrated an increasing hazard ratio for cancer mortality (all forms) at increased levels of haemoglobin acrylamide adducts, suggesting a dose-response relationship. However, there was also significant non-linearity in the association between AA-Hb levels and cancer mortality.

94. The authors also investigated the mediating effect of low-grade inflammation score (INFLA-score – based on levels of C-reactive protein, white blood cell count, platelet count, and granulocyte/lymphocyte ratio) on the association between acrylamide haemoglobin adducts and cancer mortality. The authors concluded that “the results showed that INFLA-score significantly mediated 71.67% for GA-Hb induced increased cancer mortality risk, and the direct effect of HbGA on cancer mortality showed no significance (P = 0.438), suggesting a complete mediated role of INFLA-score on the association between GA-Hb and cancer mortality.”

95. Filippini et al. (2022) performed a systematic review and dose-response meta-analysis of epidemiological studies investigating the association between dietary acrylamide exposure and several site-specific cancers. In this meta-analysis, the authors reported that high dietary acrylamide exposure was not associated with an increased risk of site-specific cancers (oral cavity, oesophageal, stomach, colorectal (including colon and rectal), pancreatic, laryngeal, lung, lymphoma, multiple myeloma, renal, bladder/urothelial, prostate, melanoma, and brain).

96. The analysis included 31 eligible papers (a total of 16 studies) with a total of 1,151,198 participants. Studies included were prospective and retrospective cohort, case-cohort and case-control studies performed in non-occupationally exposed adults (≥18 years) with acrylamide exposure assessed through diet records. Eligible studies reported hazard ratios for any type of cancer in relation to dietary acrylamide exposure, except for the female reproductive cancers (breast, endometrial, and ovarian cancers). Hazard ratios were extracted from models that adjusted for smoking status and the greatest number of other covariables.

97. Summary hazard ratios (HR) were calculated for high dietary acrylamide exposure versus low exposure for each cancer type. ‘High’ and ‘low’ acrylamide exposure levels were derived from individual studies, and for studies reporting quintiles of exposure without values, values were estimated assuming a normal distribution of exposure. Potentially non-linear associations were investigated using a cubic spline approach, with the reference value set at the mean value of acrylamide exposure (23 µg/day).

98. Summary hazard ratios demonstrated no association between high acrylamide exposure and any of the site-specific cancers. The dose-response analysis also returned a null association between acrylamide exposure at any level, and site-specific cancer. Based on their dose-response analysis, the authors suggested that associations between acrylamide exposure and site-specific cancer, “if present, may generally be without thresholds.” When studies were stratified by geographical region, a small increase in risk of lymphoma from high dietary acrylamide exposure was detected in Western populations (HR of 1.12; 95% CI 0.99-1.22). However, this analysis contained only one study.

Acrylamide induced genotoxicity in assay development

99. Several studies have also utilised the genotoxicity of acrylamide as a positive control in the development of assays with broader technological aims.

100. Barranger and Le Hégart (2022) developed a high throughput comet assay using a 3D model of HepaRG human liver cells for the assessment and prediction of genotoxic compounds. Acrylamide exposure (62.5 – 2000 µM for 48 hours) resulted in a dose dependent increase of percentage of DNA in the tail. In 3D HepaRG cultures, the BMD10 for acrylamide exposure was 25.3 µM (BMDL10 of 2.22 µM) whereas it was 215.1 µM in 2D HepaRG cultures (BMDL10 74.6 µM). The authors suggested that the lower BMDL10 in 3D cultures was due to higher expression of Cytochrome P450 (CYP) activity in these cultures, thus increasing

the metabolic conversion of acrylamide to glycidamide.

101. A study by Kuo et al. (2022) developed a pipeline for high throughput analysis of in vitro micronucleus (MNvit) assay data from 292 test compounds, including acrylamide. The main aim of their study was to develop a screening assay for classifying genotoxic agents, comparing potencies, and prioritising them for follow up. Benchmark concentrations (BMC) for 30% increases in micronucleus frequency and 60% increases in hypodiploidy were derived for each compound, and using in vitro to in vivo extrapolation (IVIVE), administered equivalent doses (AED) were calculated and compared with points of departure (NOAELs and LOAELs) from traditional in vivo toxicology studies. Cells were treated with 19 concentrations of test compounds (4.5, 5.63, 7.04, 8.8, 11, 13.7, 21.5, 26.8, 33.6, 41.9, 52.4, 65.5, 81.9, 102, 128, 160, and 200 µM) for 24 hours with and without S9. The calculated AED (mg/ kg bw/day) for acrylamide was ~100 fold higher than the point of departure from an in vivo cancer study with a NOAEL of 0.1 mg/ kg bw/day (Johnson et al. 1986). The authors suggested that acrylamide can induce cancer via non-genotoxic mechanisms, and at doses lower than those that induce genotoxicity, thus explaining this discrepancy. In validating their approach for assessing genotoxicity, they noted that the point of departure for clastogenic activity for acrylamide determined by their MNvit assay more closely aligned with points of departure derived from in vivo micronucleus assays (2- to 6- fold higher).

102. Zhang et al. (2022) developed an ultra-high performance liquid chromatography / tandem quadrupole mass spectrometry method for detection and quantification of the glycidamide adducts N7-(2-carbamoyl-2-hydroxyethyl) guanine (N7-GA-Gua) and N3-(2-carbamoyl-2-hydroxyethyl) adenine (N3-GA-Ade) in tissues and urine. Their aim was to establish the effect of catechin administration on the formation of acrylamide-induced DNA adducts. Sprague-Dawley rats were exposed to acrylamide at 1, 10 and 50 mg/kg bw and adduct levels were calculated as the sum of adducts in all urine collected over 48 hours. N7-GA-Gua and N3-GA-Ade formed in a dose-dependent manner, and the content of N7-GA-Gua (34.9–2374.4 nmol) was about 6–13 times that of N3-GA-Ade (4.4–206.3 nmol) in rat urine. The quantity of N7-GA-Gua and N3-GA-Ade in urine accounted for 1.4%–2.1 % of the total administered dose. Adducts were also detected in the liver, kidney, and heart, in a dose-dependent manner, with the highest levels detected in the kidney. Catechin (EC, EGCG or tea polyphenols) administration at 10 mg/kg bw reduced the levels of N7-GA-Gua and N3-GA-Ade in 48-h urine and tissue samples of the acrylamide exposed rats. Adduct levels were also detected in human urine from 10 participants; N7-GA-Gua was detected

in all 10 subjects, while N3-GA-Ade was only detected in 3 males and 2 females, with a lower level than N7-GA-Gua.

General toxicity

103. The literature search also returned a number of publications on non-genotoxic adverse effects of acrylamide exposure, including metabolic and immune dysfunction, inflammation, oxidative stress, and reproductive and developmental toxicity in in vitro, in vivo, and epidemiological studies. Summaries of the key findings of these studies have been included here for completion. However, it should be noted that this was not an extensive or specific literature search for these endpoints.

Cellular, metabolic, and immunological toxicity

104. Al-Hajm and Ozgun (2022) studied the effect of acrylamide exposure on protein degradation pathways in HepG2 human liver cells exposed to acrylamide at 0.01, 0.1, 1, and 10 mM. At 10 mM, acrylamide inhibited the ubiquitin proteasome system and induced autophagy.

105. In metabolomic studies of male Sprague-Dawley rats exposed to 20 mg/kg bw acrylamide, Zhao et al. (2021) observed changes in metabolites in the hippocampus, cortex, kidney, serum, heart, liver, and kidney fat. Acrylamide exposure disrupted amino acid, fatty acid, and energy metabolism and induced oxidative stress and inflammation.

106. Karimani et al. (2021) intraperitoneally administered acrylamide at 50 mg/kg bw to diabetic and non-diabetic rats for two weeks. Acrylamide exposure increased serum levels of alanine aminotransferase, blood urea nitrogen, uric acid, creatinine, and lactate dehydrogenase, and this effect was more pronounced in diabetic mice (STZ model). Acrylamide treatment also led to liver and kidney tissue lesions, which, again, was exacerbated in the diabetic mouse model.

107. Liang et al. (2022) studied the association between acrylamide exposure and hypertension and reported a significant association between GA-Hb levels and hypertension in adolescent females. GA-Hb levels also correlated with total cholesterol levels, and cholesterol levels mediated 24.2% of the association between GA-Hb and hypertension in adolescent females.

108. In a study of a nationwide US population, Wan et al. (2022) reported an association between biomarkers of acrylamide and metabolic syndrome (MetS), as defined by meeting three or more of the following criteria: elevated blood pressure, high fasting glucose, abdominal obesity, hypertriglyceridemia, and lower high-density lipoprotein cholesterol (HDL-C) levels. AA-Hb levels were inversely associated with MetS prevalence whilst the GA-Hb/AA-Hb ratio levels were significantly associated with total MetS. Acrylamide biomarkers also associated with specific features of MetS: AA-Hb was also inversely associated with hypertriglyceridemia and low HDL-C, whilst the GA-Hb/AA-Hb ratio was positively associated with abdominal obesity, hypertriglyceridemia, and low HDL-C. The authors suggested that the ratio of GA-Hb/AA-Hb levels reflects the bioactivation of acrylamide to glycidamide, and the inverse and positive associations between MetS and AA-Hb and GA-Hb/AA-Hb ratios, respectively, implicate bioactivation of acrylamide to glycidamide involvement in MetS.

109. Wu et al. (2022) investigated the associated between acrylamide exposure and mortality in people with hyperglycaemia. Levels of AA-Hb, but not GA-Hb were significantly associated with risk of cardiovascular disease mortality whilst higher AA-Hb/GA-Hb ratios were associated with increased total- and cardiovascular disease (CVD)-related mortality. Subgroup analysis showed that the highest quintile of AA-Hb/GA-Hb in people with diabetes or pre-diabetes was related to cardiovascular disease-related mortalities. The authors suggested that “the ratio of HbAA and HbGA probably reflects the balance of the detoxification metabolism in the body, and based on the findings of this study, the balance of metabolic networks for acrylamide may play a more critical role in the development of CVD among people with hyperglycaemia.”

Reproductive and developmental toxicity

110. Liver function, haematological function, and oxidative stress measures were altered in the weaned offspring of Wistar rats exposed to acrylamide during pregnancy. Tomaszewska et al. (2022) exposed pregnant Wistar rats to 3 mg/kg bw acrylamide for 5, 10 or 15 days. Prenatal acrylamide exposure affected blood morphology (reduced white blood cells, increased lymphocytes, decreased monocytes and neutrophils, and decreased red blood cell count) decreased liver mass, increased markers of liver injury, increased markers of oxidative stress, and upregulated autophagy and apoptosis in offspring. Effects were time dependent, occurring at short periods of prenatal exposure and exacerbated at longer exposure times.

111. A meta-analysis of studies investigating the association between maternal acrylamide intake and metrics of foetal growth was conducted by Hogervorst et al. (2022). Risk of being small for gestational age, lower birth weight, and lower head circumference were increased for the highest quartile of acrylamide exposure indicated by AA-Hb and GA-Hb levels. A lower birth length was only associated with maternal GA-Hb levels. The authors suggested that their findings “strengthen the evidence of an adverse effect of maternal acrylamide exposure during pregnancy on fetal growth.”

112. Meng et al. (2022) studied the association between acrylamide exposure levels and developmental disabilities in children. When modelled as continuous variables AA-Hb levels were not associated with odds of developmental disability. GA-Hb levels were associated with developmental disability, but significance was lost when the model was adjusted for covariables. When divided into quartiles, neither AA-Hb or GA-Hb levels were significantly associated with developmental disability. Restricted cubic spline analysis demonstrated a significant J-shaped association between GA-Hb, but not AA-Hb levels, and developmental disability.

Summary

113. Following a request by the EC, EFSA reassessed their Opinion on the genotoxicity of acrylamide. This request was prompted by a publication by Eisenbrand et al. (2021) that argued against a genotoxic mode of action for the carcinogenic effects of acrylamide. However, as EFSA did not consider the review by Eisenbrand to be comprehensive they conducted a literature review from the date of their previous assessment (2015) up to 2021. After scrutiny of the available evidence, EFSA upheld their 2015 Opinion on the genotoxicity of acrylamide, concluding that a margin of exposure approach was still appropriate.

114. In 2021 the Committee on Mutagenicity reviewed EFSA’s assessment and agreed with their conclusions. The current paper has reviewed EFSA’s Opinion on acrylamide, their updated assessment, and COM’s conclusions thereof. It has also provided an updated literature search on the genotoxicity of acrylamide up to mid-October 2022 which has not been reviewed by the COM. Overall, the papers returned in the literature search and summarised in the current paper do not appear to contradict EFSA’s position on the genotoxicity of acrylamide and contribute to the database on interpreting acrylamide’s mode of action.

Questions for the Committee

i. Do Members consider that the weight of evidence supports EFSA's conclusion that genotoxicity and non-genotoxic effects may contribute to the carcinogenicity of AA?

ii. Do Members agree with EFSAs conclusion that the new data does not alter the previous conclusions on the risk of AA and the MOE approach is still appropriate?

iii. Do Members consider the updated literature search to affect EFSA's conclusion on the genotoxicity of AA?

iv. Do Members have any other comments on the EFSA statement?

v. Do Members have any comments on the paper by Eisenbrand?

vi. Do Members have any other comments on the structure of the paper?

Secretariat

December 2022

Abbreviations:

µM	Micromolar
54BP1	Tumour suppressor p53-binding protein 1
AA	acrylamide
AA-Hb	Acrylamide haemoglobin adduct
AAMA	N-acetyl-S-(2- carbamoylethyl)-L-cysteine

AED	Administered equivalent dose
AFP	alpha-fetoprotein
BMC	benchmark concentration
BMDL	Benchmark dose level
BMDL	Benchmark dose lower confidence level
BMI	Body mass index
cII	Neither EFSA nor the original paper provided a definition for cII. However, the Secretariat has found the following: cII is a chromosomally integrated transcriptional activator from the bacteriophage lambda in transgenic animals. After exposure of the animals the mutant frequency can be determined via the lambda select – cII mutation detection system.
CAT	Catalase
CI	Confidence intervals
COSMIC	Catalogue of Somatic Mutations in Cancer
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
CYP	Cytochrome P450
EC	European Commission

EFSA	European Food Safety Authority
Fpg	DNA-formamidopyrimidine glycosylase
GA	glycidamide
GA-Hb	Glycidamide haemoglobin adduct
GAMA	N-(R,S)-acetyl-S-(1-carbamoyl-2-hydroxyethyl)-L-cysteine
GCL-C	Cysteine ligase catalytic subunit
gH2AX	H2A histone family member X
GI tract	Gastrointestinal tract
GSH	Glutathione
GSTA3	Glutathione S-transferase A3
GSTP-1	glutathione S-transferase
Hb	Haemoglobin
HBGV	Health based guidance value
HDAC	Histone deacetylase
HDL-C	High density lipoprotein C
HR	Hazard ratio

i.p.	Intraperitoneal injection
ICD	International Classification of Diseases
IgG	Immunoglobulin G
IgM	Immunoglobulin M
INFLA	Low-grade inflammation score
IVIVE	In vitro to in vivo extrapolation
LC50	Lethal concentration 50
LOAEL	Lowest adverse effect level
Mg/kg bw/day	milligram per kilogram body weight per day
mM	millimolar
N3	GA-Gau
N3	(2-carbamoyl-2-hydroxyethyl) adenine
N7-GA-Gau	N7-(2-carbamoyl-2-hydroxyethyl) guanine
NHANES	US National Health and Nutrition Examination Survey
NOAEL	No observed adverse effect level
PCAWG	Pan-Cancer Analysis of Whole Genomes

ROS	Reactive Oxygen species
TDI	Tolerable daily intake
THLE2	Transformed Human Liver Epithelial-2
TP53	Tumour protein 53

References

Algarni AA (2018). Genotoxic effects of acrylamide in mouse bone marrow cells. Caryologia, 71, 160–165. https://doi.org/10.1080/00087114.2018.1450801

Al-Hajm, A.Y.S., Ozgun, E., (2022). Effects of acrylamide on protein degradation pathways in human liver-derived cells and the efficacy of N-acetylcysteine and curcumin. Drug and Chemical Toxicology 45, 1536–1543. https://doi.org/10.1080/01480545.2020.1846548

Ankaiah R, Kurrey N, Krishnan MH (2018). The positive intervention effects of resveratrol on acrylamide-induced cyto-/genotoxicity in primary lymphocytes of rat. Pharmacognosy Magazine, 14, S643–S648. Available from: The positive intervention effects of resveratrol on acrylamide -induced cyto-/Genotoxicity in primary lymphocytes of rat Ankaiah R, Kurrey NK, Krishnan MH - Phcog Mag

Ansar S, Siddiqi NJ, Zargar S, Ganaie MA, Abudawood M (2016). Hepatoprotective effect of Quercetin supplementation against Acrylamide-induced DNA damage in Wistar rats. Bmc Complementary and Alternative Medicine, 16. https://doi.org/10.1186%2Fs12906-016-1322-7

Aras D, Cakar Z, Ozkavukcu S, Can A, Cinar O,= (2017). In Vivo acrylamide exposure may cause severe toxicity to mouse oocytes through its metabolite glycidamide. PLoS One, 12. https://doi.org/10.1371/journal.pone.0172026

Barranger, A., Le Hégarat, L., (2022). Towards better prediction of xenobiotic genotoxicity: CometChip technology coupled with a 3D model of HepaRG human liver cells. Arch Toxicol 96, 2087–2095. https://doi.org/10.1007/s00204-022-03292-4

Bellicha, A., Wendeu-Foyet, G., Coumoul, X., Koual, M., Pierre, F., Guéraud, F., Zelek, L., Debras, C., Srour, B., Sellem, L., Kesse-Guyot, E., Julia, C., Galan, P., Hercberg, S., Deschasaux-Tanguy, M., Touvier, M., (2022). Dietary exposure to acrylamide and breast cancer risk: results from the NutriNet-Santé cohort. Am J Clin Nutr 116, 911–919. https://doi.org/10.1093/ajcn/nqac167

Chepelev NL, Gagn_e R, Maynor T, Kuo B, Hobbs CA, Recio L, Yauk CL (2017). Transcriptional profiling of male F344 rats suggests the involvement of calcium signaling in the mode of action of acrylamide-induced thyroid cancer. Food and Chemical Toxicology, 107, 186–200. https://doi.org/10.1016/j.fct.2017.06.019

Chepelev NL, Gagn_e R, Maynor T, Kuo B, Hobbs CA, Recio L, Yauk CL (2018). Transcriptional profiling of male CD-1 mouse lungs and Harderian glands supports the involvement of calcium signalling in acrylamide-induced tumors. Regulatory Toxicology and Pharmacology, 95, 75–90. https://doi.org/10.1016/j.fct.2017.06.019

Cleusix V, Lacroix C, Vollenweider S, Le Blay G (2008). Glycerol induces reuterin production and decreases Escherichia coli population in an in vitro model of colonic fermentation with immobilized human feces. FEMS Microbiolpgy Ecology, 63, 56–64. https://doi.org/10.1111/j.1574-6941.2007.00412.x

Collí-Dula RC, Friedman MA, Hansen B, Denslow ND (2016). Transcriptomics analysis and hormonal changes of male and female neonatal rats treated chronically with a low dose of acrylamide in their drinking water. Toxicology Reports, 19, 414–426. https://doi.org/10.1016/j.toxrep.2016.03.009

David RM and Gooderham NJ (2018). Dose-dependent synergistic and antagonistic mutation responses of binary mixtures of the environmental carcinogen benzo a pyrene with food-derived carcinogens. Archives of Toxicology, 92, 3459–3469. https://doi.org/10.1007/s00204-018-2319-4

de Conti A, Tryndyak V, VonTungeln LS, Churchwell MI, Beland FA, Antunes AMM, Pogribny IP (2019). Genotoxic and epigenotoxic alterations in the lung and liver of mice induced by acrylamide: a 28 day drinking water study. Chemical Research in Toxicology, 32, 869–877. https://doi.org/10.1021/acs.chemrestox.9b00020

de Lima JP, Silva SN, Rueff J, Pingarilho M (2016). Glycidamide genotoxicity modulated by Caspases genes polymorphisms. Toxicology in Vitro, 34, 123–127. https://doi.org/10.1016/j.tiv.2016.03.018

Dobrovolsky VN, Pacheco-Martinez MM, McDaniel LP, Pearce MG, Ding W (2016). In vivo genotoxicity assessment of acrylamide and glycidyl methacrylate. Food and Chemical Toxicology, 87, 120–127. https://doi.org/10.1016/j.fct.2015.12.006

EFSA (2015). Scientific Opinion on acrylamide in food. EFSA Journal 2015;13(6):4104, 321 pp. https://doi.org/10.2903/j.efsa.2015.4104

EFSA (2022). Assessment of the genotoxicity of acrylamide. EFSA Journal, 20(5): 7293. https://doi.org/10.2903/j.efsa.2022.7293

Eisenbrand G (2020a). Revisiting the evidence for genotoxicity of acrylamide (AA), key risk assessment of dietary AA exposure. Archives of Toxicology, 94, 2399–2950. https://doi.org/10.1007/s00204-020-02794-3

Eisenbrand G (2020b). Correction to: Revisiting the evidence for genotoxicity of acrylamide (AA), key to risk assessment of dietary AA exposure. Archives of Toxicology, 94, 3935. https://doi.org/10.1007%2Fs00204-020-02893-1

Filippini, T., Halldorsson, T.I., Capitão, C., Martins, R., Giannakou, K., Hogervorst, J., Vinceti, M., Åkesson, A., Leander, K., Katsonouri, A., Santos, O., Virgolino, A., Laguzzi, F., (2022). Dietary Acrylamide Exposure and Risk of Site-Specific Cancer: A Systematic Review and Dose-Response Meta-Analysis of Epidemiological Studies. Front Nutr 9, 875607. https://doi.org/10.3389/fnut.2022.875607

Goempel K, Tedsen L, Ruenz M, Bakuradze T, Schipp D, Galan J, Eisenbrand G, Richling E (2017). Biomarker monitoring of controlled dietary acrylamide exposure indicates consistent human endogenous background. Archives of Toxicology, 91, 3551–3560. https://doi.org/10.1007/s00204-017-1990-1

Gouveia-Fernandes, S., Rodrigues, A., Nunes, C., Charneira, C., Nunes, J., Serpa, J., Antunes, A.M.M., (2022). Glycidamide and cis-2-butene-1,4-dial (BDA) as potential carcinogens and promoters of liver cancer - An in vitro study. Food Chem Toxicol 166, 113251. https://doi.org/10.1016/j.fct.2022.113251

Gu, W., Zhang, J., Ren, C., Gao, Y., Zhang, T., Long, Y., Wei, W., Hou, S., Sun, C., Wang, C., Jiang, W., Zhao, J., (2022). The association between biomarkers of acrylamide and cancer mortality in U.S. adult population: Evidence from NHANES 2003-2014. Front Oncol 12, 970021. https://doi.org/10.3389/fonc.2022.970021

Hagio S, Tsuji N, Furukawa S, Takeuchi K, Hayashi S, Kuroda Y, Honma M, Masumura K (2021). Effect of sampling time on somatic and germ cell mutations induced by acrylamide in gpt delta mice. Genes and Environment, 43, 4.

https://doi.org/10.1186/s41021-021-00175-5

Hansen SH, Pawlowicz AJ, Kronberg L, Gutzkow KB, Olsen AK, Brunborg G (2018). Using the comet assay and lysis conditions to characterize DNA lesions from the acrylamide metabolite glycidamide. Mutagenesis, 33, 31–39. https://doi.org/10.1093/mutage/gex036

Hashem, M.M., Abo-EL-Sooud, K., Abd El-Hakim, Y.M., Abdel-hamid Badr, Y., El-Metwally, A.E., Bahy-EL-Dien, A., (2022). The impact of long-term oral exposure to low doses of acrylamide on the hematological indicators, immune functions, and splenic tissue architecture in rats. International Immunopharmacology 105, 108568. https://doi.org/10.1016/j.intimp.2022.108568

Hemgesberg M, Stegmüuller S, Cartus A, Schrenk D (2021a). A Benchmark analysis of acrylamide-derived DNA adducts in rat hepatocytes in culture measured by a new, highly sensitive method. Toxicology, 464, 153022. https://doi.org/10.1016/j.tox.2021.153022

Hemgesberg M, Stegmüller S, Cartus A, Hemmer S, Püttmann M, Stockis JP, Schrenk D (2021b). Acrylamide derived DNA adducts in human peripheral blood mononuclear cell DNA: correlation with body mass. Food and Chemical Toxicology, 112575. https://doi.org/10.1016/j.fct.2021.112575

Hobbs CA, Davis J, Shepard K, Chepelev N, Friedman M, Marroni D, Recio L (2016). Differential genotoxicity of acrylamide in the micronucleus and pig-a gene mutation assays in F344 rats and B6C3F1 mice. Mutagenesis, 31, 617–626. https://doi.org/10.1093/mutage/gew028

Hoelzl-Armstrong L, Kucab JE, Moody S, Zwart EP, Loutkotov_a L, Duffy V, Luijten M, Gamboa da Costa G, Stratton MR, Phillips DH, Arlt VM (2020a). Mutagenicity of acrylamide and glycidamide in human TP53 knock-in (Hupki) mouse embryo fibroblasts. Archives of Toxicology, 94, 4173–4196. https://doi.org/10.1007/s00204-020-02878-0

Hogervorst, J., Virgolino, A., Halldorsson, T.I., Vinceti, M., Åkesson, A., Leander, K., Nawrot, T., Filippini, T., Laguzzi, F., (2022). Maternal acrylamide exposure during pregnancy and fetal growth: A systematic review and dose-response meta-analysis of epidemiological studies. Environ Res 213, 113705. https://doi.org/10.1016/j.envres.2022.113705

Holzl-Armstrong L, Nævisdal A, Cox JA, Long AS, Chepelev NL, Phillips DH, White PA, Arlt VM (2020b). In vitro mutagenicity of selected environmental carcinogens

and their metabolites in MutaMouse FE1 lung epithelial cells. Mutagenesis, 35, 453–463. https://doi.org/10.1093/mutage/geaa032

Horibata K, Ukai A, Honma M (2016). Evaluation of mutagenicity of acrylamide using RBC Pig-a and PIGRET assays by single peroral dose in rats. Mutation Research-Genetic Toxicology and Environmental Mutagenesis, 811, 54–59. https://doi.org/10.1016/j.mrgentox.2015.12.005

Huang CC, Wu CF, Shih WC, Luo YS, Chen MF, Li CM, Liou SH, Chung WS, Chiang SY, Wu KY (2015). Potential association of urinary N7-(2-carbamoyl-2-hydroxyethyl) guanine with dietary acrylamide intake of smokers and non-smokers. Chemical Research in Toxicology, 28, 43–50. https://doi.org/10.1021/tx500265p

Huang YF, Huang CJ, Lu CA, Chen ML, Liou SH, Chiang SY, Wu KY (2018). Feasibility of using urinary N7-(2-carbamoyl-2-hydroxyethyl) Guanine as a biomarker for acrylamide exposed workers. Journal of Exposure Science and Environmental Epidemiology, 28, 589–598. https://doi.org/10.1038/s41370-018-0018-0

IARC (International Agency for Research on Cancer), 1994. IARC Monographs on the Evaluations of Carcinogenic Risks to Humans. Volume 60. Some Industrial Chemicals. Acrylamide. Summary of data reported and Evaluation. Last updated: 13 April 1999.

Ishi Y, Matsushita K, Kuroda K, Yokoo Y, Kijima A, Takasu S, Kodama Y, Nishikawa A, Umemura T (2015). Acrylamide induces specific DNA adduct formation and gene mutations in a carcinogenic target site, the mouse lung. Mutagenesis, 30, 227–235. https://doi.org/10.1093/mutage/geu062

Jangir BL, Mahaprabhu R, Rahangadale S, Bhandarkar AG, Kurkure NV (2016). Neurobehavioral alterations and histopathological changes in brain and spinal cord of rats intoxicated with acrylamide. Toxicology and Industrial Health, 32, 526–540. https://doi.org/10.1177/0748233713505893

Johnson, K.A., Gorzinski, S.J., Bodner, K.M., Campbell, R.A., Wolf, C.H., Friedman, M.A., Mast, R.W., (1986). Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. Toxicol Appl Pharmacol 85, 154–168. https://doi.org/10.1016/0041-008x(86)90109-2

Jones DJL, Singh R, Emms V, Farmer PB, Grant D, Quinn P, Maxwell C, Mina A, Ng LL, Schumacher S, Britton RG (2021). Determination of N7-glycidamide guanine

adducts in human blood DNA following exposure to dietary acrylamide using liquid chromatography/tandem mass spectrometry. Rapid Communications in Mass Spectrometry, RCM, 36, e9245. https://doi.org/10.1002/rcm.9245

Karimani, A., Hosseinzadeh, H., Mehri, S., Jafarian, A.H., Kamali, S.A., Hooshang Mohammadpour, A., Karimi, G., (2021). Histopathological and biochemical alterations in non-diabetic and diabetic rats following acrylamide treatment. Toxin Reviews 40, 277–284. https://doi.org/10.1080/15569543.2019.1566263

Katen AL, Chambers CG, Nixon B, Roman SD (2016b). Chronic acrylamide exposure in male mice results in elevated DNA damage in the germline and heritable induction of CYP2E1 in the testes. Biology of Reproduction, 95, 1–15. https://doi.org/10.1095/biolreprod.116.139535

Katen AL, Stanger SJ, Anderson AL, Nixon B, Roman SD (2016a). Chronic acrylamide exposure in male mice induces DNA damage to spermatozoa: Potential for amelioration by resveratrol. Reproductive Toxicology, 63, 1–12. https://doi.org/10.1016/j.reprotox.2016.05.004

Komiya M, Ishigamori R, Naruse M, Ochiai M, Miyoshi N, Imai T, Totsuka Y (2021). Establishment of novel genotoxicity assay system using murine normal epithelial tissue-derived organoids. Frontiers in Genetics, 12, 768781. https://doi.org/10.3389/fgene.2021.768781

Kontaş Yedier, S., Atlı Şekeroğlu, Z., Şekeroğlu, V., Aydın, B., (2022). Cytotoxic, genotoxic, and carcinogenic effects of acrylamide on human lung cells. Food and Chemical Toxicology 161, 112852. https://doi.org/10.1016/j.fct.2022.112852

Kuo, B., Beal, M.A., Wills, J.W., White, P.A., Marchetti, F., Nong, A., Barton-Maclaren, T.S., Houck, K., Yauk, C.L., (2022). Comprehensive interpretation of in vitro micronucleus test results for 292 chemicals: from hazard identification to risk assessment application. Arch Toxicol 96, 2067–2085. https://doi.org/10.1007/s00204-022-03286-2

Kuo, L.J., Yang, L.-X., (2008). Gamma-H2AX - a novel biomarker for DNA double-strand breaks. In Vivo 22, 305–309. Available at: γ-H2AX - A Novel Biomarker for DNA Double-strand Breaks | In Vivo (iiarjournals.org)

Li HF, Shelton SD, Townsend TA, Mei N, Manjanatha MG (2016). Evaluation of cII gene mutation in the brains of Big Blue mice exposed to acrylamide and glycidamide in drinking water. Journal of Toxicological Sciences, 41, 719–730. https://doi.org/10.2131/jts.41.719

Liang, J., Xu, C., Liu, Q., Weng, Z., Zhang, X., Xu, J., Gu, A., (2022). Total cholesterol: a potential mediator of the association between exposure to acrylamide and hypertension risk in adolescent females. Environ Sci Pollut Res Int 29, 38425–38434. https://doi.org/10.1007/s11356-021-18342-0

Liu S, Jiang L, Zhong T, Kong S, Zheng R, Kong F, Zhang C, Zhang L, An L (2015). Effect of acrylamide on oocyte nuclear maturation and cumulus cells apoptosis in mouse in vitro. PLoS One, 10. https://doi.org/10.1371/journal.pone.0135818

Liu Z, Wang J, Chen S, Xu C, Zhang Y (2021). Associations of acrylamide with non-alcoholic fatty liver disease in American adults: a nationwide cross-sectional study. Environmental Health, 20, 98. https://doi.org/10.1186/s12940-021-00783-2

Mah, L.-J., El-Osta, A., Karagiannis, T.C., (2010). γH2AX: a sensitive molecular marker of DNA damage and repair. Leukemia 24, 679–686. https://doi.org/10.1038/leu.2010.6

Mandon M, Huet S, Dubreil E, Fessard V, Le Hegarat L (2019). Three-dimensional HepaRG spheroids as a liver model to study human genotoxicity in vitro with the single cell gel electrophoresis assay. Scientific Reports, 9, 10548. https://doi.org/10.1038/s41598-019-47114-7

Meng, F., Qi, Y., Wu, Y., He, F., (2022). Association between acrylamide exposure and the odds of developmental disabilities in children: A cross-sectional study. Frontiers in Public Health 10. https://doi.org/10.3389/fpubh.2022.972368

Nowak A, Zakłos-Szyda M, Z_ yz_elewicz D, Koszucka A, Motyl I (2020). Acrylamide decreases cell viability, and provides oxidative stress, DNA damage, and apoptosis in human colon adenocarcinoma cell line caco-2. Molecules, 25, 368. https://doi.org/10.3390/molecules25020368

NTP (2012). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Acrylamide (CAS No. 79-06-1) in F344/N rats and B6C3F1 mice (feed and drinking water studies). NTP TR 575. NIH Publication No. 12-5917. National Institutes of Health. Public Health Service. U.S. Department of Health and Human Services. July 2012.

Panier, S., Boulton, S.J., (2014). Double-strand break repair: 53BP1 comes into focus. Nat Rev Mol Cell Biol 15, 7–18. https://doi.org/10.1038/nrm3719

Ruenz M, Bakuradze T, Eisenbrand G, Richling E (2016). Monitoring urinary mercapturic acids as biomarkers of human dietary exposure to acrylamide in

combination with acrylamide uptake assessment based on duplicate diets. Archives in Toxicology, 90, 873–881. https://doi.org/10.1007/s00204-015-1494-9

Ruenz M, Goerke K, Bakuradze T, Abraham K, Lampen A, Eisenbrand G, Richling E (2019). Sustained human background exposure to acrolein evidenced by monitoring urinary exposure biomarkers. Molecular Nutrition and Food Research, 63, e1900849. https://doi.org/10.1002/mnfr.201900849

Salimi A, Baghal E, Ghobadi H, Hashemidanesh N, Khodaparast F, Seydi E (2021a). Mitochondrial, lysosomal and DNA damages induced by acrylamide attenuate by ellagic acid in human lymphocyte. PLoS One, 16, e0247776. https://doi.org/10.1371/journal.pone.0247776

Salimi A, Hashemidanesh N, Seydi E, Baghal E, Khodaparast F, Ghobadi H (2021b). Restoration and stabilization of acrylamide-induced DNA, mitochondrial damages and oxidative stress by chrysin in human lymphocyte. Expert Opinion on Drug Metabolism & Toxicology, 17, 857–865. https://doi.org/10.1080/17425255.2021.1940951

Sekeroglu ZA, Aydın B, Sekeroglu V (2017). Argan oil reduces oxidative stress, genetic damage and emperipolesis in rats treated with acrylamide. Biomedicine and Pharmacotherapy, 94, 873–879. https://doi.org/10.1016/j.biopha.2017.08.034

Shimamura Y, Iio M, Urahira T, Masuda S (2017). Inhibitory effects of Japanese horseradish (Wasabia japonica) on the formation and genotoxicity of a potent carcinogen, acrylamide. Journal of the Science of Food and Agriculture, 97, 2419–2425. https://doi.org/10.1002/jsfa.8055

Tareke E, Lyn-Cook B, Robinson B, Ali SF (2008). Acrylamide: a dietary carcinogen formed in vivo? Journal of Agricultural and Food Chemistry, 56, 6020–6023. https://doi.org/10.1021/jf703749h

Tomaszewska, E., Muszyński, S., Świetlicka, I., Wojtysiak, D., Dobrowolski, P., Arciszewski, M.B., Donaldson, J., Czech, A., Hułas-Stasiak, M., Kuc, D., Mielnik-Błaszczak, M., (2022). Prenatal acrylamide exposure results in time-dependent changes in liver function and basal hematological, and oxidative parameters in weaned Wistar rats. Sci Rep 12, 14882. https://doi.org/10.1038/s41598-022-19178-5

Wan, X., Zhu, F., Zhuang, P., Liu, X., Zhang, L., Jia, W., Jiao, J., Xu, C., Zhang, Y., (2022). Associations of Hemoglobin Adducts of Acrylamide and Glycidamide with Prevalent Metabolic Syndrome in a Nationwide Population-Based Study. J. Agric.

Food Chem. 70, 8755–8766. https://doi.org/10.1021/acs.jafc.2c03016

Wang B, Qiu W, Yang S, Cao L, Zhu C, Ma J, Li W, Zhang Z, Xu T, Wang X, Cheng M, Mu G, Wang D, Zhou Y, Yuan J, Chen W (2020). Acrylamide exposure and oxidative DNA damage, lipid peroxidation, and fasting plasma glucose alteration: association and mediation analyses in Chinese urban adults. Diabetes Care, 43, 1479–1486. https://doi.org/10.2337/dc19-2603

Wang ET, Chen DY, Liu HY, Yan HY, Yuan Y (2015). Protective effect of allicin against glycidamide-induced toxicity in male and female mice. General Physiology and Biophysics, 34, 177–187. https://doi.org/10.4149/gpb_2014038

Wang P, Ji R, Ji J, Chen F (2019). Changes of metabolites of acrylamide and glycidamide in acrylamide-exposed rats pretreated with blueberry anthocyanins extract. Food Chemistry, 274, 611–619. https://doi.org/10.1016/j.foodchem.2018.08.058

Wang RS, McDaniel LP, Manjanatha MG, Shelton SD, Doerge DR, Mei N (2010). Mutagenicity of acrylamide and glycidamide in the testes of big blue mice. Toxicological Sciences, 117, 72–80. https://doi.org/10.1093/toxsci/kfq190

Wang SS, Wang H, Chen Y, Liu J, He X, Huang D, Wu Y, Chen Y, Weng Z (2021). Protective effects of (-)-epigallocatechin gallate and curcumin against acrylamide toxicity. Toxicological and Environmental Chemistry, 103, 199–218. https://doi.org/10.1080/02772248.2021.1951731

Wu, H., Sun, X., Jiang, H., Hu, C., Xu, J., Sun, C., Wei, W., Han, T., Jiang, W., (2022). The Association Between Exposure to Acrylamide and Mortalities of Cardiovascular Disease and All-Cause Among People With Hyperglycemia. Front Cardiovasc Med 9, 930135. https://doi.org/10.3389/fcvm.2022.930135

Xiao D, Wang H, Han D (2016). Single and combined genotoxicity effects of six pollutants on THP-1 cells. Food and Chemical Toxicology, 95, 96–102. https://doi.org/10.1016/j.fct.2016.06.029

Zamani E, Shokrzadeh M, Modanloo M, Shaki F (2018). In vitro study towards role of acrylamide-induced genotoxicity in human lymphocytes and the protective effect of L-Carnitine. Brazilian Archives of Biology and Technology, 61, e18160685. https://doi.org/10.1590/1678-4324-20181600685

Zhang J, Sturla S, Lacroix C, Schwab C (2018). Gut Microbial Glycerol Metabolism as an Endogenous Acrolein Source, MBio, 9, e01947–e2017.

https://doi.org/10.1128/mbio.01947-17

Zhang, Yiju, Wang, Q., Li, Y., Cheng, J., Chen, X., Zhang, Yu, (2022). Comprehensive profile of DNA adducts as both tissue and urinary biomarkers of exposure to acrylamide and chemo-preventive effect of catechins in rats. Chemosphere 286, 131852. https://doi.org/10.1016/j.chemosphere.2021.131852

Zhao M, Liu X, Luo Y, Guo H, Hu X, Chen F (2015a). Evaluation of protective effect of freeze-dried strawberry, grape, and blueberry powder on acrylamide toxicity in mice. Journal of Food Science, 80, H869–H874. https://doi.org/10.1111/1750-3841.12815

Zhao M, Wang P, Zhu Y, Liu X, Hu X, Chen F (2015b). Blueberry anthocyanins extract inhibits acrylamide induced diverse toxicity in mice by preventing oxidative stress and cytochrome P450 2E1 activation. Journal of Functional Foods, 14, 95–101. https://doi.org/10.1016/j.jff.2015.01.035

Zhao, S., Zhong, H., Geng, C., Xue, H., Wang, C., Sun, W., Dang, R., Han, W., Jiang, P., (2021). Comprehensive analysis of metabolic changes in rats exposed to acrylamide. Environmental Pollution 287, 117591. https://doi.org/10.1016/j.envpol.2021.117591

Zhivagui M, Ng AWT, Ardin M, Churchwell MI, Pandey M, Renard C, Villar S, Cahais V, Robitaille A, Bouaoun L, Heguy A, Guyton KZ, Stampfer MR, McKay J, Hollstein M, Olivier M, Rozen SG, Beland FA, Korenjak M, Zavadil J (2019). Experimental and pan-cancer genome analyses reveal widespread contribution of acrylamide exposure to carcinogenesis in humans. Genome Research, 29, 521–531. https://doi.org/10.1101/gr.242453.118

TOX/2022/66 Annex A

Literature search on the genotoxicity of acrylamide

Background

1. Following the publication of an article that questioned the genotoxic nature of acrylamide (AA), (Eisenbrand, 2020) EFSA provided a scientific statement to assess whether an update on the scientific opinion on acrylamide in food was required. In preparing their assessment, EFSA conducted a literature

search on the genotoxicity of AA for the period from the publication of the CONTAM statement (2015) to the end of 2021. EFSA's assessment supported the conclusions of the 2015 statement published by the CONTAM.

2. The aim of the current search was to assess whether any new relevant studies on the genotoxic potential of AA have been published since EFSA conducted their latest search.

Methods

3. EFSA conducted a literature search using Web of Science which encompasses the following databases: Web of Science Core Collection; BIOSIS Citation Index; CABI: CAB Abstracts; Current Contents Connect; Data Citation Index; FSTA – the food science resource; MEDLINE; SciELO Citation Index; Zoological Record. The search strings they used, the date of the search, and the years they encompassed are as follows:

Type	Search terms	years	Date
Genotoxicity of AA	Acrylamide AND genotoxicity	2015-2021	24/9/2021
Adducts of AA or GA	Acrylamide OR glycidamide AND adducts	2015-2021	9/12/2021

Epidemiological studies	Acrylamide AND Cross-sectional OR cohort OR case-control AND Cancer OR carcinogenicity OR tumours	2015-2021	17/11/2021

4. The search for this current paper used the same search strings employed by EFSA using Web of Science and an additional search in PubMed. For each string, results were refined to reflect articles published from the end date of the EFSA search up to mid-October 2022.

Results

Summary

5. Each search string returned several results, and some results were redundant (i.e., identified by more than one search string). PubMed and Web of Science returned partially non-overlapping results, highlighting the importance of scoping both databases. However, for the epidemiology search string, PubMed returned a large number of studies about chemotherapeutic compounds, which required manual exclusion. The full results and citations for each search string are listed at the end of this appendix.

6. A minority of results were relevant for the current paper and are summarised in Annex B. The majority of the remaining results fell into one of several general classes not directly investigating the relationship between acrylamide exposure and genotoxicity and/or carcinogenesis. These classes were: entirely irrelevant (i.e., not focused on acrylamide as a toxin/contaminant), the EFSA assessment, review articles, analysis of acrylamide in food, exposure assessments, non-genotoxic adverse effects of acrylamide, toxicokinetic studies, development/validation of bioassays, and detection/analysis of acrylamide in food.

7. One study that was primarily focused on non-genotoxic adverse effects of acrylamide (Hashem et al., 2022) also performed a comet assay analysis. Several of the bioassay development studies provided data on the genotoxic potential of acrylamide. These studies were included in the summaries in Annex B under ‘genotoxicity/carcinogenesis’ and are also listed below.

8. A number of studies investigating non-genotoxic effects of acrylamide that were retrieved in the search were also summarised although this was not a comprehensive search for other toxicological endpoints. These included in vitro, in vivo, and epidemiology studies. Studies investigating non-genotoxic endpoints in non-mammalian systems, or that were primarily focused on therapeutic/interventional measures were excluded from the summaries.

9. The distribution of results amongst these classes for the three search terms is summarised below in Table 1.

Table 1. Summary of search results by search term and category of study.

Type	EFSA	Reviews	EA	Food	Non-genotoxic
Genotoxicity	1	4	4	4	10
Adducts	0	3	8	0	12
Epidemiology	0	0	2	0	0

Type	Bioassays	Irrelevant	TK	Genotoxic	Other	Total
Genotoxicity	5	3	0	3	0	34
Adducts	3	3	2	3	0	34
Epidemiology	0	0	0	0	3	5

EFSA=EFSA assessment; EA=exposure assessment; TK=toxicokinetics.

10. A detailed breakdown of the results, and an indication of relevant studies are outlined below. Directly relevant studies are listed, along with studies for which brief summaries were provided, including bioassay development and validation, and studies reporting non-genotoxic adverse effects of AA.

Genotoxicity of AA

11. Combined unique articles from Web of Science and PubMed search for “Acrylamide AND genotoxicity” returned 34 results. The full results are listed at the end of this document.

12. Of these results, three results were irrelevant to the search (i.e., not focused on AA as a contaminant/toxin). One was the EFSA assessment, and four results were review articles. Four articles were focused on AA exposure assessment, four were focused on AA in food and 10 were focused on non-genotoxic adverse effects of AA. Of the remaining seven studies, five were focused on developing and/or validating bioassays for detecting AA-induced genotoxicity, and three were mechanistic studies investigating AA-induced genotoxicity (one of which (Hashem et al., 2022) did not investigate genotoxicity as the primary endpoint). The studies for which summaries were provided are listed below in Tables 2 - 4.

Table 2. Studies assessing genotoxicity/carcinogenic potential of acrylamide.

Author	**Title**	**Journal**
Gouveia-Fernandes et al. (2022)	Glycidamide and cis-2-butene-1,4-dial (BDA) as potential carcinogens and promoters of liver cancer – An in vitro study	Food and Chemical Toxicology
Kontaş Yedier et al. (2022)	Cytotoxic, genotoxic, and carcinogenic effects of acrylamide on human lung cells	Food and Chemical Toxicology

Hashem et al. (2022)	The impact of long-term oral exposure to low doses of acrylamide on the hematological indicators, immune functions, and splenic tissue architecture in rats	International Immunopharmacology

Table 3. Studies using AA-induced genotoxicity in assay development and validation.

Author	**Title**	**Journal**
Barranger et al. (2022)	Towards better prediction of xenobiotic genotoxicity: CometChip technology coupled with a 3D model of HepaRG human liver cells	Archives of Toxicology
Kuo et al. (2022)	Comprehensive interpretation of in vitro micronucleus test results for 292 chemicals: from hazard identification to risk assessment application	Archives of Toxixology
Zhang et al. (2022)	Comprehensive profile of DNA adducts as both tissue and urinary biomarkers of exposure to acrylamide and chemo-preventive effect of catechins in rats	Chemosphere

Table 4. Studies on general AA toxicity.

Author	**Title**	**Journal**
Karimani et al. (2021)	Histopathological and biochemical alterations in non-diabetic and diabetic rats following acrylamide treatment	Toxin Reviews

Zhao et al. (2021)	Comprehensive analysis of metabolic changes in rats exposed to acrylamide	Environmental Pollution

Adducts of AA or GA

13. Combined unique articles from Web of Science and PubMed search for "(Acrylamide OR glycidamide) AND adducts" returned 47 unique results. 13 results were co-hits from the "genotoxicity of AA" search, giving 34 new results. The full list of (unique) results is at the end of this document.

14. Of these results, three were irrelevant to the search (i.e., not focused on AA as a contaminant/toxin). Three results were review articles, two were focused on toxicokinetics of AA/GA, eight were focused on exposure/dietary assessment, 12 were focused on non-genotoxic adverse effects of AA/GA and three studies developed bioassays for detection/quantification of AA and/or its biomarkers. The three remaining studies were focused on AA exposure and cancer risk in humans. The studies for which summaries were provided are listed below in Tables 5 and 6.

Table 5. Studies assessing genotoxicity/carcinogenic potential of acrylamide.

Author	**Title**	**Journal**
Bellicha et al. (2022)	Dietary exposure to acrylamide and breast cancer risk: results from the NutriNet-Sante cohort	American Journal of Clinical Nutrition
Filippini et al. (2022)	Dietary Acrylamide Exposure and Risk of Site-Specific Cancer: A Systematic Review and Dose-Response Meta-Analysis of Epidemiological Studies	Frontiers in Nutrition
Gu et al. (2022)	The association between biomarkers of acrylamide and cancer mortality in U.S. adult population: Evidence from NHANES 2003-2014	Frontiers in Oncology

Table 6. Studies on general AA toxicity.

Author	Title	Journal
Al-Hajm et al. (2022)	Effects of acrylamide on protein degradation pathways in human liver-derived cells and the efficacy of N-acetylcysteine and curcumin	Drug and Chemical Toxicology
Hogervorst et al. (2022)	Maternal acrylamide exposure during pregnancy and fetal growth: A systematic review and dose-response meta-analysis of epidemiological studies	Drug and Chemical Toxicology
Liang et al. (2022)	Total cholesterol: a potential mediator of the association between exposure to acrylamide and hypertension risk in adolescent females	Environmental Science and Pollution Research
Meng et al. (2022)	Association between acrylamide exposure and the odds of developmental disabilities in children: A cross-sectional study	Frontiers in Public Health
Tomaszewska et al. (2022)	Prenatal acrylamide exposure results in time-dependent changes in liver function and basal hematological, and oxidative parameters in weaned Wistar rats	Scientific Reports
Wan et al. (2022)	Associations of Hemoglobin Adducts of Acrylamide and Glycidamide with Prevalent Metabolic Syndrome in a Nationwide Population-Based Study	Journal of Agricultural and Food Chemistry

Wu et al. (2022)	The Association Between Exposure to Acrylamide and Mortalities of Cardiovascular Disease and All-Cause Among People with Hyperglycemia	Frontiers in Cardiovascular Medicine

Epidemiological Studies

15. Combined unique articles from Web of Science and PubMed search for “Acrylamide AND (cross-sectional OR cohort OR case-control) AND (cancer OR carcinogenicity OR tumours)” returned 60 unique results. The majority (52) of these results were about chemotherapy with cancer-preventing drugs that contain acrylamide as a functional component of the molecule and were therefore manually excluded from the results. Of the remaining eight studies, three results (Bellicha et al. 2022; Filippini et al., 2022 and Kontaş Yedier et al., 2022) were co-hits from the other two searches, giving five new results. These results are listed at the end of this document.

16. Two of these studies were about general dietary xenobiotics, one was focused on the association between volatile organic compounds and liver injury, one was about the association between ultra-processed foods and colorectal cancer, and one was about the effect of coffee/tea/caffeine in breast cancer. None of the studies were directly relevant to assessing genotoxicity/carcinogenic potential of acrylamide per se.

Conclusions

17. Since EFSA conducted their updated literature search to review the genotoxicity of acrylamide, a large number of additional studies have been published. Whilst many of these studies were not considered relevant for the current purpose (being review articles, exposure assessments, etc.) a smaller number may be relevant for assessing the genotoxic potential of acrylamide. Five of these studies (three mechanistic and two epidemiological) affirm the association between acrylamide and genotoxicity and/or carcinogenesis, three studies used AA-induced genotoxicity as a positive control in assay development and validation, whilst one meta-analysis did not find a dose-response relationship between acrylamide exposure and site-specific cancers.

Full search results (number of articles)

Acrylamide AND genotoxicity (34)

Mechanistic studies on genotoxicity (3)

Gouveia-Fernandes, S. et al. (2022) ‘Glycidamide and cis-2-butene-1,4-dial (BDA) as potential carcinogens and promoters of liver cancer - An in vitro study’, Food and Chemical Toxicology, 166. Available at: https://doi.org/10.1016/j.fct.2022.113251.

Kontaş Yedier, S. et al. (2022) ‘Cytotoxic, genotoxic, and carcinogenic effects of acrylamide on human lung cells.’, Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association, 161, p. 112852. Available at: https://doi.org/10.1016/j.fct.2022.112852.

Hashem, M.M. et al. (2022) ‘The impact of long-term oral exposure to low doses of acrylamide on the hematological indicators, immune functions, and splenic tissue architecture in rats’, International Immunopharmacology, 105. Available at: https://doi.org/10.1016/j.intimp.2022.108568.

Bioassays and detection (5)

Barranger, A. and Le Hégarat, L. (2022) ‘Towards better prediction of xenobiotic genotoxicity: CometChip technology coupled with a 3D model of HepaRG human liver cells.’, Archives of toxicology, 96(7), pp. 2087–2095. Available at: https://doi.org/10.1007/s00204-022-03292-4.

Gauch, F., Abraham, K. and Monien, B.H. (2022) ‘Simultaneous quantification of eight hemoglobin adducts of genotoxic substances by isotope-dilution UHPLC-MS/MS.’, Analytical and bioanalytical chemistry, 414(19), pp. 5805–5815. Available at: https://doi.org/10.1007/s00216-022-04143-y.

Harahap, Y. et al. (2022) ‘Analysis of Acrylamide and Glycidamide in Dried Blood Spot of Smokers Using Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry.’, Drug design, development and therapy, 16, pp. 521–531. Available at: https://doi.org/10.2147/DDDT.S346892.

Kuo, B. et al. (2022) ‘Comprehensive interpretation of in vitro micronucleus test results for 292 chemicals: from hazard identification to risk assessment application’, Archives of Toxicology, 96(7), pp. 2067–2085. Available at:

https://doi.org/10.1007/s00204-022-03286-2.

Zhang, Yiju et al. (2022) ‘Comprehensive profile of DNA adducts as both tissue and urinary biomarkers of exposure to acrylamide and chemo-preventive effect of catechins in rats’, Chemosphere, 286(3). Available at: https://doi.org/10.1016/j.chemosphere.2021.131852.

Non-genotoxic adverse effects of AA (10)

Atamanalp, M. et al. (2022) ‘Borax relieved the acrylamide-induced hematotoxic, hepatotoxic, immunotoxic and genotoxic damages in rainbow trout by regulating apoptosis and Nrf2 signaling pathway’, comparative biochemistry and physiology C- toxicology & pharmacology, 259. Available at: https://doi.org/10.1016/j.cbpc.2022.109396.

Erkmen, B. et al. (no date) ‘Sublethal effects of acrylamide on thyroid hormones, complete blood count and micronucleus frequency of vertebrate model organism (Cyprinus carpio)’, Turkish Journal of Biochemistry Turk Biyokimya Dergisi [Preprint]. Available at: https://doi.org/10.1515/tjb-2022-0025.

Haridevamuthu, B. et al. (2022) ‘Amelioration of acrylamide induced neurotoxicity by benzo[b]thiophene analogs via glutathione redox dynamics in zebrafish larvae’, BRAIN RESEARCH, 1788. Available at: https://doi.org/10.1016/j.brainres.2022.147941.

Karimani, A. et al. (2021) ‘Histopathological and biochemical alterations in non-diabetic and diabetic rats following acrylamide treatment’, Toxin Reviews, 40(3), pp. 277–284. Available at: https://doi.org/10.1080/15569543.2019.1566263.

Kovár, M. et al. (2022) ‘Acrylamide-Derived Ionome, Metabolic, and Cell Cycle Alterations Are Alleviated by Ascorbic Acid in the Fission Yeast.’, Molecules (Basel, Switzerland), 27(13). Available at: https://doi.org/10.3390/molecules27134307.

Ligina, V. et al. (no date) ‘Acute and sublethal effects of acrylamide on the freshwater fish Anabas testudineus (Bloch, 1792)’, Environmental Science and Pollution Research [Preprint]. Available at: https://doi.org/10.1007/s11356-022-22155-0.

Wang, J. et al. (2022) ‘The inhibitory effect of Yam polysaccharides on acrylamide-induced programmed cell death in RAW 264.7 cells’, Food Science & Nutrition [Preprint]. Available at: https://doi.org/10.1002/fsn3.3076.

Wang, A. et al. (2022) ‘Catechins protect against acrylamide- and glycidamide-induced cellular toxicity via rescuing cellular apoptosis and DNA damage.’, Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, 167, p. 113253. Available at: https://doi.org/10.1016/j.fct.2022.113253.

Yesildag, K. et al. (2022) ‘Evaluation of the protective effects of morin against acrylamide-induced lung toxicity by biomarkers of oxidative stress, inflammation, apoptosis, and autophagy.’, Journal of food biochemistry, 46(7), p. e14111. Available at: https://doi.org/10.1111/jfbc.14111.

Zhao, S. et al. (2021) ‘Comprehensive analysis of metabolic changes in rats exposed to acrylamide.’, Environmental pollution (Barking, Essex : 1987), 287, p. 117591. Available at: https://doi.org/10.1016/j.envpol.2021.117591.

Acrylamide in food (4)

Kim, J., Nedwidek-Moore, M. and Kim, K. (2022) ‘Safest Roasting Times of Coffee To Reduce Carcinogenicity’, Journal of Food Protection, 85(6), pp. 918–923. Available at: https://doi.org/10.4315/JFP-21-427.

Li, X. et al. (2021) ‘Low Acrylamide Flatbreads from Colored Corn and Other Flours.’, Foods (Basel, Switzerland), 10(10). Available at: https://doi.org/10.3390/foods10102495.

Monsalve-Atencio, R., Montaño, D.F. and Contreras-Calderón, J. (2022) ‘Molecular imprinting technology and poly (ionic liquid)s: Promising tools with industrial application for the removal of acrylamide and furanic compounds from coffee and other foods.’, Critical reviews in food science and nutrition, pp. 1–20. Available at: https://doi.org/10.1080/10408398.2022.2038078.

Paranthaman, R., Moses, J.A. and Anandharamakrishnan, C. (2022) ‘Novel powder-XRD method for detection of acrylamide in processed foods.’, Food research international (Ottawa, Ont.), 152, p. 110893. Available at: https://doi.org/10.1016/j.foodres.2021.110893.

Exposure/dietary assessment (4)

Costa, S.A. et al. (2022) ‘Risk characterization of dietary acrylamide exposure and associated factors in the Portuguese population.’, Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment, 39(5), pp. 888–900. Available at: https://doi.org/10.1080/19440049.2022.2047540.

Esposito, F. et al. (2022) ‘Acrylamide levels in smoke from conventional cigarettes and heated tobacco products and exposure assessment in habitual smokers.’, Environmental research, 208, p. 112659. Available at: https://doi.org/10.1016/j.envres.2021.112659.

Nica-Badea, D. (2022) ‘Relevance of dietary exposure to acrylamide formed in heat-processed agri-food products.’, Central European journal of public health, 30(3), pp. 179–184. Available at: https://doi.org/10.21101/cejph.a6779.

Rietjens, I.M.C.M. et al. (2022) ‘The role of endogenous versus exogenous sources in the exposome of putative genotoxins and consequences for risk assessment.’, Archives of toxicology, 96(5), pp. 1297–1352. Available at: https://doi.org/10.1007/s00204-022-03242-0.

Reviews (4)

Iyer, A.M., Dadlani, V. and Pawar, H.A. (2022) ‘Review on Acrylamide: A Hidden Hazard in Fried Carbohydrate-rich Food’, Current Nutrition & Food Science, 18(3), pp. 274–286. Available at: https://doi.org/10.2174/1573401318666220104124753 .

Kopańska, M. et al. (2022) ‘Acrylamide Neurotoxicity as a Possible Factor Responsible for Inflammation in the Cholinergic Nervous System.’, International journal of molecular sciences, 23(4). Available at: https://doi.org/10.3390/ijms23042030.

Kumar, S.S. et al. (no date) ‘A systematic review on the effects of acrylamide and bisphenol A on the development of Drosophila melanogaster’, Molecular Biology Reports [Preprint]. Available at: https://doi.org/10.1007/s11033-022-07642-4.

Pietropaoli, F. et al. (2022) ‘Acrylamide in widely consumed foods - a review.’, Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment, 39(5), pp. 853–887. Available at: https://doi.org/10.1080/19440049.2022.2046292.

EFSA assessment (1)

Benford, D. et al. (2022) ‘Assessment of the genotoxicity of acrylamide’, EFSA Journal, 20(5). Available at: https://doi.org/10.2903/j.efsa.2022.7293.

Irrelevant (3)

Furusawa, G. and Iwamoto, K. (2022) ‘Removal of Microcystis aeruginosa cells using the dead cells of a marine filamentous bacterium, Aureispira sp. CCB-QB1’, PEERJ, 10. Available at: https://doi.org/10.7717/peerj.12867.

Hasan, S. and Naeem, A. (2021) ‘The modulation of structural stability of horseradish peroxidase as a consequence of macromolecular crowding.’, Journal of molecular recognition : JMR, 34(10), p. e2902. Available at: https://doi.org/10.1002/jmr.2902.

Li, J., How, Z.T. and Gamal El-Din, M. (2022) ‘Aerobic degradation of anionic polyacrylamide in oil sands tailings: Impact factor, degradation effect, and mechanism.’, The Science of the total environment, 856(Pt 1), p. 159079. Available at: https://doi.org/10.1016/j.scitotenv.2022.159079.

(Acrylamide OR glycidamide) AND adducts (34)

AA and cancer risk (3)

Bellicha, A. et al. (2022) ‘Dietary exposure to acrylamide and breast cancer risk: results from the NutriNet-Sante cohort’, American Journal of Clinical Nutrition [Preprint]. Available at: https://doi.org/10.1093/ajcn/nqac167.

Filippini, T. et al. (2022) ‘Dietary Acrylamide Exposure and Risk of Site-Specific Cancer: A Systematic Review and Dose-Response Meta-Analysis of Epidemiological Studies’, Frontiers in Nutrition, 9. Available at: https://doi.org/10.3389/fnut.2022.875607.

Gu, W. et al. (2022) ‘The association between biomarkers of acrylamide and cancer mortality in U.S. adult population: Evidence from NHANES 2003-2014.’, Frontiers in oncology, 12, p. 970021. Available at: https://doi.org/10.3389/fonc.2022.970021.

Bioassays and detection (3)

Gan, Z. et al. (2022) ‘Ratiometric Fluorescent Metal-Organic Framework Biosensor for Ultrasensitive Detection of Acrylamide.’, Journal of agricultural and food chemistry, 70(32), pp. 10065–10074. Available at: https://doi.org/10.1021/acs.jafc.2c04756.

Liu, Y. et al. (2022) ‘A fluorescence biosensor based on double-stranded DNA and a cationic conjugated polymer coupled with exonuclease III for acrylamide detection.’, International journal of biological macromolecules, 219, pp. 346–352.

Available at: https://doi.org/10.1016/j.ijbiomac.2022.07.251.

Shimamura, Y. et al. (2021) ‘Factors Influencing the Formation of Chemical-Hemoglobin Adducts.’, Toxics, 10(1). Available at: https://doi.org/10.3390/toxics10010002.

Non-genotoxic adverse effects of AA/GA (12)

Al-Hajm, A.Y.S. and Ozgun, E. (2022) ‘Effects of acrylamide on protein degradation pathways in human liver-derived cells and the efficacy of N-acetylcysteine and curcumin.’, Drug and chemical toxicology, 45(4), pp. 1536–1543. Available at: https://doi.org/10.1080/01480545.2020.1846548.

Chen, M. et al. (2022) ‘Formation and metabolism of 6-(1-acetol)-8-(1-acetol)-rutin in foods and in vivo, and their cytotoxicity.’, Frontiers in nutrition, 9, p. 973048. Available at: https://doi.org/10.3389/fnut.2022.973048.

Filipovic, J.M. et al. (2022) ‘Acrylamide and Potential Risk of Diabetes Mellitus: Effects on Human Population, Glucose Metabolism and Beta-Cell Toxicity’, International Journal of Molecular Sciences, 23(11). Available at: https://doi.org/10.3390/ijms23116112.

Hogervorst, J. et al. (2022) ‘Maternal acrylamide exposure during pregnancy and fetal growth: A systematic review and dose-response meta-analysis of epidemiological studies.’, Environmental research, 213, p. 113705. Available at: https://doi.org/10.1016/j.envres.2022.113705.

Li, D. et al. (2022) ‘Formation and Identification of Six Amino Acid - Acrylamide Adducts and Their Cytotoxicity Toward Gastrointestinal Cell Lines.’, Frontiers in nutrition, 9, p. 902040. Available at: https://doi.org/10.3389/fnut.2022.902040.

Liang, J. et al. (2022) ‘Total cholesterol: a potential mediator of the association between exposure to acrylamide and hypertension risk in adolescent females’, Environmental Science and Pollution Research, 29(25), pp. 38425–38434. Available at: https://doi.org/10.1007/s11356-021-18342-0.

Meng, F. et al. (2022) ‘Association between acrylamide exposure and the odds of developmental disabilities in children: A cross-sectional study.’, Frontiers in public health, 10, p. 972368. Available at: https://doi.org/10.3389/fpubh.2022.972368.

Tomaszewska, E. et al. (2022) ‘Prenatal acrylamide exposure results in time-dependent changes in liver function and basal hematological, and oxidative

parameters in weaned Wistar rats', Scientific Reports, 12(1). Available at: https://doi.org/10.1038/s41598-022-19178-5.

Wan, X., Jia, W., et al. (2022) 'Metabolomics strategy comprehensively unveils the effect of catechins intervention on the biomarkers of exposure to acrylamide and biomarkers of cardiometabolic risk.', Environment international, 169, p. 107517. Available at: https://doi.org/10.1016/j.envint.2022.107517.

Wan, X., Zhu, F., et al. (2022) 'Associations of Hemoglobin Adducts of Acrylamide and Glycidamide with Prevalent Metabolic Syndrome in a Nationwide Population-Based Study.', Journal of agricultural and food chemistry, 70(28), pp. 8755–8766. Available at: https://doi.org/10.1021/acs.jafc.2c03016.

Wu, H. et al. (2022) 'The Association Between Exposure to Acrylamide and Mortalities of Cardiovascular Disease and All-Cause Among People with Hyperglycemia.', Frontiers in cardiovascular medicine, 9, p. 930135. Available at: https://doi.org/10.3389/fcvm.2022.930135.

Yin, T. et al. (2022) 'Vitamin D mediates the association between acrylamide hemoglobin biomarkers and obesity.', Environmental science and pollution research international, 29(12), pp. 17162–17172. Available at: https://doi.org/10.1007/s11356-021-16798-8.

Exposure/dietary assessment (8)

Cheng, Y.-W. et al. (2022) 'Health risks of dual use of electronic and combustible cigarettes: exposure to acrylamide and glycidamide', Polish Archives of Internal Medicine Polskie Archiwum Medycyny Wewnetrznej,132(1). Available at: https://doi.org/10.20452/pamw.16103.

Jones, D.J.L. et al. (2022) 'Determination of N7-glycidamide guanine adducts in human blood DNA following exposure to dietary acrylamide using liquid chromatography/tandem mass spectrometry.', Rapid communications in mass spectrometry: RCM, 36(6), p. e9245. Available at: https://doi.org/10.1002/rcm.9245.

Kenwood, B.M. et al. (2022) 'Cigarette smoking is associated with acrylamide exposure among the US population: NHANES 2011-2016', Environmental Research, 209. Available at: https://doi.org/10.1016/j.envres.2022.112774.

Liao, K.-W. et al. (2022) 'Associating acrylamide internal exposure with dietary pattern and health risk in the general population of Taiwan', FOOD CHEMISTRY,

374. Available at: https://doi.org/10.1016/j.foodchem.2021.131653.

Poteser, M. et al. (2022) ‘Time Trends of Acrylamide Exposure in Europe: Combined Analysis of Published Reports and Current HBM4EU Studies.’, Toxics, 10(8). Available at: https://doi.org/10.3390/toxics10080481.

Poteser, M., Laguzzi, F., Schettgen, T., Vogel, N., Weber, T., Murawski, A., et al. (2022) ‘Trends of Exposure to Acrylamide as Measured by Urinary Biomarkers Levels within the HBM4EU Biomonitoring Aligned Studies (2000-2021)’, TOXICS, 10(8). Available at: https://doi.org/10.3390/toxics10080443.

Sabbioni, G. et al. (2022) ‘Literature review and evaluation of biomarkers, matrices and analytical methods for chemicals selected in the research program Human Biomonitoring for the European Union (HBM4EU).’, Environment international, 169, p. 107458. Available at: https://doi.org/10.1016/j.envint.2022.107458.

Scherer, G., Pluym, N. and Scherer, M. (no date) ‘Comparison of urinary mercapturic acid excretions in users of various tobacco/nicotine products’, Drug Testing and Analysis [Preprint]. Available at: https://doi.org/10.1002/dta.3372.

Toxicokinetics (2)

Karimani, A., Karimi, G. and Mohammadpour, A.H. (no date) ‘Acrylamide and glycidamide in plasma of diabetic and non-diabetic rats, a comparative toxicokinetic study’, Toxicological and Environmental Chemistry [Preprint]. Available at: https://doi.org/10.1080/02772248.2022.2104280.

Li, Y. et al. (2022) ‘The construction and application of physiologically based toxicokinetic models for acrylamide, glycidamide and their biomarkers in rats and humans.’, Chemosphere, 292, p. 133458. Available at: https://doi.org/10.1016/j.chemosphere.2021.133458.

Reviews (3)

Albiach-Delgado, A. et al. (2022) ‘Review of the state of the art of acrylamide human biomonitoring’, Chemosphere, 295. Available at: https://doi.org/10.1016/j.chemosphere.2022.133880.

Pedersen, M. et al. (2022) ‘Hemoglobin adducts of acrylamide in human blood - What has been done and what is next?’, Food and chemical toxicology: an international journal published for the British Industrial Biological Research

Association, 161, p. 112799. Available at: https://doi.org/10.1016/j.fct.2021.112799.

Zhao, M., Zhang, B. and Deng, L. (2022) 'The Mechanism of Acrylamide-Induced Neurotoxicity: Current Status and Future Perspectives', Frontiers in Nutrition, 9. Available at: https://doi.org/10.3389/fnut.2022.859189.

Irrelevant (3)

Hooper, A.R. and Burns, A.S. (2022) 'Thermal Stability and Utility of Dienes as Protecting Groups for Acrylamides.', ACS medicinal chemistry letters, 13(5), pp. 833–840. Available at: https://doi.org/10.1021/acsmedchemlett.2c00075.

Kumler, M.S. et al. (no date) 'Trials and adventures of the synthesis and evaluation of amphiphilic graft copolymers with dynamic topology', JOURNAL OF POLYMER SCIENCE [Preprint]. Available at: https://doi.org/10.1002/pol.20220247.

Muste, C. and Gu, C. (2022) 'BTK-inhibitor drug covalent binding to lysine in human serum albumin using LC-MS/MS.', Drug metabolism and pharmacokinetics, 42, p. 100433. Available at: https://doi.org/10.1016/j.dmpk.2021.100433.

Epidemiological studies (5)

Ruiz-Saavedra, S. et al. (2022) 'Dietary Xenobiotics Derived from Food Processing: Association with Fecal Mutagenicity and Gut Mucosal Damage', NUTRIENTS, 14(17). Available at: https://doi.org/10.3390/nu14173482.

Wahlang, B. et al. (2021) 'Associations Between Residential Exposure to Volatile Organic Compounds and Liver Injury Markers.', Toxicological sciences: an official journal of the Society of Toxicology, 185(1), pp. 50–63. Available at: https://doi.org/10.1093/toxsci/kfab119.

Wang, L. et al. (2022) 'Association of ultra-processed food consumption with colorectal cancer risk among men and women: results from three prospective US cohort studies', BMJ-BRITISH MEDICAL JOURNAL, 378. Available at: https://doi.org/10.1136/bmj-2021-068921.

Wang, S. et al. (2021) 'Does coffee, tea and caffeine consumption reduce the risk of incident breast cancer? A systematic review and network meta-analysis', Public Health Nutrition, 24(18), pp. 6377–6389. Available at: https://doi.org/10.1017/S1368980021000720.

Zapico, A. et al. (2022) ‘Pilot Study for the Dietary Assessment of Xenobiotics Derived from Food Processing in an Adult Spanish Sample’, FOODS, 11(3). Available at: https://doi.org/10.3390/foods11030470.